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GEORGE RICHARD LYMAN
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Recording the main facts of a man's existence, and his passing, is a relatively simple task. To interpret and express the influence which the personality moving in that existence had on his fellows is incomparably more difficult. Influences are elusive and intangible; kindliness, sincerity, and unwavering adherence to ideals too soon become shadows of the memory; they are never adequately portrayed.

George Richard Lyman was born on December 1, 1871, at Lee Center, Illinois, and died at Baltimore, Maryland, on June 7, 1926. His parents were George A. and Mary E. (Jones) Lyman. On June 23, 1903, he was married to Frances E. Badger, of Amboy, Illinois. Mrs. Lyman and their one daughter, Mavis, and his aged mother, survive him.

During his boyhood Dr. Lyman lived on a grain and stock farm in Northern Illinois. When 18 years old he went away for further training than the local schools could give; and in 1894 he received his A.B. degree from Beloit College. He then became superintendent of schools at Amboy, Illinois, for two years. He resigned to do graduate work at Harvard University. After securing an A.B. degree in 1897 and an A.M. in 1899, he continued his studies there while acting as Austin Teaching Fellow in Cryptogamic Botany from 1897 to 1900, and as Instructor in Botany in Radcliffe College in 1900–1901. In 1901 he went to Dartmouth College as Instructor in Botany; and in 1904 he was made Assistant Professor of Biology (Botany). He held this title at the time of his resignation from Dartmouth in January, 1915. In June, 1906, he received a Ph.D. degree from Harvard. During the school year 1912–13 he was on leave from Dartmouth, and, in Dr. Thaxter's absence, took charge of the work in cryptogamic botany at Harvard, with the title of Lecturer. His summers from 1906 to 1914 were spent at the Marine Biological Laboratory, Woods Hole, as one of the staff of instructors in the course on the morphology and physiology of the Algae.

He entered the service of the United States Department of Agriculture on February 1, 1915, for the first month and a half dividing his time between the Office of Forest Pathology and the Federal Horticultural Board. On March 16 of the same year, however, he was transferred to a full time position as a pathological inspector in the Federal Horticultural Board; and in this service he took an increasingly valuable and responsible part until July 31, 1917, when he assumed the direction of the newly-created Plant Disease Survey.

On January 15, 1923, he resigned from the Department of Agriculture to become, on January 21, Administrative Head in Charge of the Agricultural Experiment Station, the Division of Agricultural Extension, and the instructional work in the College of Agriculture of West Virginia.

Dr. Lyman was a member of Beta Theta Pi and of Phi Beta Kappa. He was elected to the American Association for the Advancement of Science (Sections G and O) in 1906, and made a Fellow in 1909; he was vice-president of Section G (Botany) in 1924. He was a member of the Botanical Society of America, the American Society of Naturalists, the Botanical Society of Washington, and the Washington Academy of Sciences; he was a Fellow of the American Academy of Arts and Sciences.

His membership in the American Phytopathological Society began in 1914; and he became a life sustaining member in 1920. He was elected secretary-treasurer of the society and business manager of PHYTOPATHOLOGY at the Baltimore meeting, December, 1918, and served until December, 1922. Dr. Lyman took office at an exceedingly difficult time; war prices prevailed for printing, as well as everything else, and were still mounting. The journal was going into debt an increasing amount each year; its poor financial condition caused him a great deal of worry. His gratitude when the members of the Society contributed practically half of the indebtedness facing the Society at the time of the Chicago meeting was a deeply personal thing which indicated how close was the bond between the Society and himself. A change in the methods of handling the journal had become imperative, and after much consideration he made the change in 1920. It resulted in a much improved condition for PHYTOPATHOLOGY but it threw on him additional duties connected with the management of subscriptions, advertising, and sales, which up to that time had been handled by the publishers.

The War Emergency Board originated at the Pittsburgh meeting, December, 1917; and he was the representative on the Board from the Department of Agriculture. He put a great deal of time and effort into the work and was one of the most useful members of the Board. His counsel was always sought, and the members soon discovered his ability, good judgment, and wisdom. When the War Emergency Board was succeeded by the Advisory Board, he was elected chairman. He also served as the Society's

representative in the Division of Biology and Agriculture of the National Research Council from 1919 to 1922.

He was president of the Society for 1923.

Thus can be set down the chief mile stones in his journey; those of us who walked with him even a short space have a vivid recollection of the man in him that made the journey unforgetable. Very early in his career Dr. Lyman won the confidence of his associates. In college he took his place as a leader in the affairs of his fraternity and in the activities of the college as a whole. The same qualities which had characterized him from the first developed steadily throughout his work as student, teacher, investigator and executive. His personality was singularly distinct; and the elements which made it so were many. Courteous simplicity, open honesty, and an incredible lack of cynicism were combined with gentle humor, kindness, patience, and sincerity. These qualities were the foundation on which he builded his success and influence.

His deep interest in his subject and his painstakingly clear presentation of it naturally aroused in certain of his students an eagerness for further study in some phase of botany. As the years went on, an appreciable number of men were thus led into their life activity. Some devoted themselves to teaching, some to government service, some to forestry, and some to commercial botany. They were as different as the phases of work they had taken up and as the various schools of training in which they had pursued their graduate work; but all of them shared one thing in common—an enthusiasm which Dr. Lyman had transmitted to them and awakened in them, and a love and respect for the man who had started them in their profession.

He was one of the clearest and most effective lecturers we have ever heard. It required considerable mental and manual agility to keep up with him, for one could not safely omit as unimportant any point which he brought forward; his notes had been culled of the uncontributive and unimportant before he started to talk. Although essentially patient and tolerant, he could be effectively severe when necessary. In the laboratory he taught his students the value of adequate records and impartial interpretation, insisting on the utmost attainable accuracy in observation and representation of the things studied.

He was not restricted to books and laboratory, for he knew and taught plants as they grew. By nature a keen observer, collector and field investigator, he had studied widely different groups of plants—algae, fungi, trees and flowering plants—in their natural surroundings; and he made them all vital and interesting in his fascinating courses. The field trips which he conducted were by no means picnics. Rather, during them a great deal of very effective teaching was done in the open. Despite a general attitude of

informality, much was demanded and much was accomplished in response to his demand. Actively he led, here, there, and everywhere at once, scooping a bottle of slimy mixture from a green and odorous pool, pointing out the gross differences between a *Russula* and a *Lactarius,* suggesting aptly that an old log had better be rolled over—there might be some fine Myxomycetes on its underside.

There was a directness about his methods which accomplished pedagogical wonders within the hours available for such work. At times a puckish twinkle in his eyes would reveal the saving and sympathetic humor underlying his academic seriousness. "This, gentlemen, is *Arisaema* and, as you note, the corm is especially developed for storage. Would you, Mr. Husis, care to taste it?" He would, and did. After all there was something about *Arisaema* which made one remember it! Perhaps it was evolved just to break up the tenseness toward the end of a field trip. In any case the demonstration was successful.

In his outdoor work he always showed himself a thorough sportsman. He was an unusually able walker. Many an athlete, swinging a mile or two back to the college at a four-mile-an-hour rate, after a field trip that had covered several miles through rough and rugged country, learned to look at the spare but vigorous figure with increasing respect. He was never unduly censorious if a convenient snow-ball in winter or a snake or toad in spring were slipped deftly into the gaping collar or pocket of the intent and unwary by those whose high spirits led them to activities that embellished the more serious intellectual work in hand.

The quality so essential in a teacher of being patient with those who were just stepping out on the path along which he had gone so far was carried into all phases of his life's work. In dealing with hard-headed individuals or with pompous gentlemen of more eloquence than knowledge, who sometimes gathered to protest against various measures, his patience and understanding kindliness were always unaltered and undiminished. Dr. Lyman was distinguished by his obvious sincerity. If he urged a student to improve his drawing, it was because he truly believed that the student should better himself; if he urged the destruction of a shipment of valuable but dangerously infected plant material, the disgruntled investigator for whom the shipment was destined realized that there was no false pretense back of his recommendation; if he appealed for greater funds for carrying on some phase of departmental work, there stood out in his appeal—even in a letter or memorandum—the unselfish conviction that the appropriation was necessary for the betterment of the service. All who came in contact with him felt this sincerity, and, as was inevitable, it communicated itself to others; no one was associated with him in any phase of academic or departmental activity without being in some measure influenced by it.

In Dr. Lyman's work as an investigator there may be traced the same qualities that characterized him as a teacher. His doctor's thesis represents a painstaking, conscientious piece of investigation in a very difficult field, where there was so little promise of reward that a less earnest or persevering worker would have given up, discouraged, long before the first results were obtained. Moreover, its completion was materially retarded by the heavy load of teaching which he assumed at Dartmouth in 1901. In spite of such obstacles it was finally made ready and published in February, 1907. In the years that followed, only a few occasional papers appeared. The thesis on the polymorphism of the Hymenomycetes, a very able piece of work, remains his only major contribution to mycological literature.

During his academic service his creative efforts went into the preparation of his lectures, his reports, his plans of organization or procedure, outlines of the course work, and keys for the identification of fungi, flowers, shrubs, and trees. His key to the trees around Hanover, N. H., a small pamphlet used in one of his classes at Dartmouth, is still one of the most practical and useful manuals of its kind which we have ever encountered. One of us still treasures a manuscript of some 30 pages of certain parts of Saccardo's keys, which he painstakingly copied years ago as an aid in his study and teaching. Lantern slides, material, and charts used by him at Dartmouth and Harvard still bear his neatly written labels; schedules for laboratory work and field trips are pinned on the wall of his office at Woods Hole—mute evidence of the care that he took in even the most minute details of his work.

The record of Dr. Lyman's years in the Department of Agriculture is so recent, his success as an organizer and an administrator there are so well known that little need be written here. There was a vast difference between the duties of a quiet academic position and the executive work of the Federal Horticultural Board which involved varied contacts not only with investigators, but also with growers, shippers, and business men. An unusual amount of seasoned executive ability appeared to be required. At first sight his success and progress seem surprising. A relatively short time showed clearly how effectively the teacher could adapt himself to his new surroundings. The ability which had enabled him to keep pace with the expanding department of botany at Dartmouth enabled him also to meet the demands made on him by the rapid development of the pathological inspection work, and by the active growth of the Plant Disease Survey.

He had remarkable ability as a mediator and pacificator. For example, his efforts in the dispute over the proposed location of the potato wart quarantine zone in Pennsylvania in 1920 straightened out what might have been

a very serious situation. Conditions which irritated others aroused in him a sort of amused tolerance that rendered him particularly effective in difficult situations. Disputes, professional or quasi-political, were quietly settled; his knowledge of the actual scientific facts involved, combined with liberal common sense and human appreciation of the widely varied points of view which were represented, won over even the most unreasonable.

Under his able direction the Plant Disease Survey became an active organization of collaborators who reported plant disease conditions in their respective regions and cooperated with the Department in special survey projects. The machinery for mapping epidemics and for estimating their cost to the country was set in motion, and kept running with great efficiency. In a few years, he who had held a relatively isolated professorship had built up a phytopathological organization of nation-wide importance, and become an authority on the problems with which it dealt. There can be no better expression of appreciation of this service than the following paragraph from the letter written by Dr. W. A. Taylor, chief of the Bureau of Plant Industry, to acknowledge Dr. Lyman's resignation:

"The development of the Plant Disease Survey work has required unusual tact, patience and perseverance, qualities which we have all been gratified to recognize as possessed by you and stimulated in your associates through the influence of your example. It has been a class of work of pioneer character in the pathological field and we feel that you have rendered a service of very great value, not only to this department, but to science generally, where too frequently in the past individualistic tendency has prevented or unfortunately retarded constructive cooperative effort."

With his call to West Virginia University there came to Dr. Lyman opportunity to continue his activities as an executive and administrator in an even more varied field and to take up again his academic work as a teacher and leader. That the three years allotted to him there were productive ones is evidenced by the esteem in which he was held by the college and by the extension and experiment station staffs. That his work in the future would have been even more valuable and important is certain.

The news that Dr. Lyman was in poor health reached us in November, 1925, just at a time when we were visioning for him a hope picture of a particularly bright future. The serious nature of his illness became apparent after an operation at Sibley Hospital, Washington, on December 14. A second operation at Johns Hopkins Hospital on May 6, 1926, served only to confirm the diagnosis of cancer and to reveal the hopelessness of his case. With characteristic fortitude and patience he went through the days that followed. On June 6 he appeared, as one friend has written us, "so like Doctor Lyman of old"; but very early on the morning of June 7 the un-

equal struggle ended. Funeral services were held on June 10 at Morgantown, West Virginia, and some days later the interment took place at Dixon, Illinois.

It is impossible to write of him without mention of what was so large, so vital, and so fundamentally a part of his life—his love for his family. His utter considerateness toward Mrs. Lyman and toward his daughter Mavis was a keynote in his character. Their suffering was his and their pleasure his deepest enjoyment; in their difficulties or triumphs they were inexpressibly close to his heart; invariably it was his custom to place them and their happiness before himself and his own interests.

His botanical contributions may be overshadowed by the results of more comprehensive investigations; his achievements in various offices at Washington may be superseded by the activities of others; but some measure of his ideals will persist and extend the influence of his personality through his students and associates.

To the best of our knowledge the following is a complete list of Dr. Lyman's publications:

Culture studies on the polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. **33**[4]: 125–209. 1907.

Keys to the trees of Hanover, New Hampshire, based on winter characters and on leaves. Issued by Dartmouth College, 1909.

The trees and shrubs of Hanover, New Hampshire. Issued by Dartmouth College, 1911.

Keys to the fungi of Hanover, New Hampshire. Privately issued, 1912.

Outlines of cryptogamic botany. Privately issued, 1914.

The native habitat of *Spongospora subterranea*. Science **42**: 940–941. 1915.

The need for organization of American botanists for more effective prosecution of war work. Science **47**: 279–284. 1918.

The relation of phytopathologists to plant disease survey work. Phytopath. **8**: 219–228. 1918.

The unification of American botany. Science **49**: 339–345. 1919.

The Advisory Board of American Plant Pathologists. Phytopath. **9**: 202–206. 1919.

Potato wart—a dangerous new disease. U. S. Dept. Agr. Circ. 32. 1919.

Potato wart. U. S. Dept. Agr. Circ. 111. 1920.

Reports and summaries on distribution, severity, etc., of plant diseases in the United States. Plant Disease Bulletin. U. S. Dept. Agr. 1917–1923.

Reports of the Annual Meetings of the American Phytopathological Society. Phytopath. **10**: 264–271. 1920; **11**: 194–204. 1921; **12**: 195–204. 1922; **13**: 188–198. 1923.

PHENOMENA ASSOCIATED WITH THE DESTRUCTION OF THE CHLOROPLASTS IN TOMATO MOSAIC[1]

HELEN SOROKIN[2]

INTRODUCTION

Despite the fact that leaf mottling is one of the most conspicuous symptoms of the mosaic diseases, very little attention has been paid in modern literature to the actual process by which the chloroplasts are destroyed in the chlorotic regions of mosaic leaves. In the general search for the pathogene of this peculiar disease, various pathological cell inclusions, like protoplasmic bodies attached to the nuclei, or various motile "protein bodies," attracted most of the attention of investigators.

The present paper describes certain pathological inclusions found in mosaic affected cells of tomato leaves, but its main purpose is to describe the disintegration of the chloroplasts, and to try to explain this process from the point of view of changes which might occur in such a colloidal system as a living cell.

The observation of living material has been the principal method of the investigation. The fixing of free-hand sections on the slide under the microscope was practiced to a considerable extent, and cytological technique served only for some supplementary purposes, as well as for the histological observations. As a fixing reagent in this latter case, Merkel's fluid was found to be the most suitable. The sections were stained with haematoxylin and with safranin and gentian-violet.

HISTOLOGICAL CHANGES IN MOSAIC TISSUE

Woods (34) and Iwanowski (12) established the fact that there are definite and typical changes in the structure of mosaic leaves of tobacco. The most conspicuous of these were the failure of the tissues in the chlorotic

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station as paper No. 691 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The investigation was undertaken as a cooperative project between the Section of Plant Pathology and the Section of Plant Physiology.

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regions to differentiate into palisade and spongy parenchyma, and the development of more or less isodiametric cells. Histological studies on tomato were made by Westerdijk (33), Melchers (24) and Dickson (4). Westerdijk did not find any striking differentiation between the yellow and the adjoining green or healthy areas in mosaic tomato leaves, except that the yellow regions were sometimes only slightly thinner. Somewhat similar results were obtained by Melchers. Dickson, on the contrary, showed that distinct histological abnormalities occur in severe cases of mosaic. Sections through the chlorotic regions showed the presence of cuboidal palisade cells, while sections through the dark areas of mosaic tomato leaves had perfectly developed, elongated palisade cells. In less severe cases of the disease the histological changes were not so pronounced, but still there was a reduction in the length of the cells. Furthermore, Dickson (4) brought forward some additional evidence for the general conclusion that the lighter green areas in many mosaic diseases are characterized by hypoplasia of palisade and spongy parenchyma, as well as by the reduction of the intercellular space volume in these tissues.

The writer's observations of the histological changes in tomato leaves affected with mosaic are in complete agreement with the conclusions of Dickson. A section through a decidedly chlorotic region of a young leaf in a severe stage of mosaic disease is represented in Plate XII, A. All cells are nearly isodiametric; there is no differentiation into palisade and spongy parenchyma; the intercellular space volume is very limited; the epidermal cells are hardly distinguishable from the parenchyma cells. The chlorotic regions in less severely diseased leaves are characterized by the fact that the first row of parenchyma cells are slightly longer than those of the other three or four rows. In other respects the condition is similar to that which is represented in Plate XII, A. As to the cell contents, almost all of the essential components of a normal cell are lacking or modified. The nuclei are often absent or only about half of their normal size. Chloroplasts are absent entirely in a great number of the cells. In other cells few modified plastids could be observed. Groups of transparent vesicles often occupy the cell, or it may be vacuolated. The deep green portions, on the contrary, have normally developed palisade cells (Pl. XII, B). The spongy parenchyma and intercellular spaces are present and the epidermal cells are somewhat hypertrophied. A transitional zone between the two described regions is shown in Plate XII, C. In many of the cells of this region the chloroplasts are absent or replaced by transparent vesicles. In other cells the plastids are present. There often is a granular substance in cells which are devoid of the normal cell components.

PREVIOUS STUDIES ON THE CAUSE OF CHLOROSIS IN MOSAIC TISSUES

The studies of different authors on the structure of the chloroplasts in chlorotic regions of different plants affected with mosaic have mostly been made on fixed material. A generally accepted opinion is that the chloroplasts are fewer, paler and smaller in the chlorotic regions than in normal tissues. Köning (14) was the first to call attention to the disorganization of chloroplasts in mosaic tobacco plants. He also observed a general disorganization of the leaf tissues. Westerdijk (33) says that in yellow areas of the mosaic tomato leaves the chloroplasts are yellowish, slightly smaller than normal, and contain but little starch. Again, Melchers (24) found no difference in the number or size of the chloroplasts from the chlorotic and green areas in tomato leaves. Doolittle (5) found that the chloroplasts in the cells of cucumber affected with mosaic were smaller than in the normal cells, and were often pressed so closely to the walls of the cell as to be almost invisible. Kunkel (15) explains the occurrence of the lighter regions in corn mosaic as due to the failure of those portions to develop the normal green color rather than to a fading out of the green color after it has been produced. Kunkel (17) states that it is possible to observe the chlorotic areas in the process of formation in mosaic sugar cane. It consists in the coalescence of scattered, small chlorotic spots into a larger spot. A decrease both in number and in size of the chloroplasts has been observed by Cook (1) in the chlorotic regions of mosaic sugar cane. He explains this condition as being a result of under-development rather than disintegration. Rawlins and Johnson (30) state that the chlorotic appearance in mottled mosaic leaves of tobacco is due to a failure of the palisade cells of these plants to elongate.

Iwanowski (12) studied the changes in the chloroplasts of living cells in the chlorotic regions in mosaic tobacco. He found that the chloroplasts were very scarce, often were either swollen or liquefied or changed to peculiar vesicles with a thin green rim. These vesicles sometimes occupied the whole cell (Iwanowski's fig. 7). Dickson (4) made numerous observations of the chloroplasts in living cells in the chlorotic regions of the different mosaic plants. His general conclusions are that the chloroplasts are fewer, paler, often broken down, coalesced and degenerated. According to him, this latter process is as follows: The chloroplasts lose their green color and break up into many small, hyaline granules of varying size, which move rapidly. The bodies become brownish when treated with iodine-potassium iodide.

THE DESTRUCTION OF CHLOROPLASTS IN MOSAIC TISSUES

In the present investigation, a process of the actual dissolution of the proteins of the chloroplasts has been observed in the chlorotic regions of the

diseased leaves. This process was accompanied by peculiar changes in the general aspect of the plastids. For the observation of the above mentioned phenomenon in the living condition, thin sections were made from the surface of the transitional zone between the green and chlorotic regions of mosaic leaves. The sections were cut parallel to the leaf surface in such a way as to leave the epidermal cells and the first row of the parenchyma cells untouched. These sections were mounted in water, and were observed by focusing through the epidermal cells. Apochromatic, oil immersion lenses were used. A camera lucida drawing of part of one of these sections is represented in Plate XIII, A. In the two cells at the extreme upper left of this drawing there are numerous apparently healthy chloroplasts. In a homogenous stroma are included granules and droplets (Pl. XIII, C, 1). Hyaline bodies, which were surrounded by a halo (Pl. XIII, C, 2), were found in certain chloroplasts from the green area, and in very many chloroplasts from the transitional zone between the green and chlorotic areas. The bodies measured about 2 μ in length and were stationary. In certain other chloroplasts the halo was absent and the hyaline bodies were actively revolving (Pl. XIII, C, 3). If viewed from one side, the bodies appeared like elongated crystals with one edge somewhat pointed and the other slightly rounded (Pl. XIII, E, 1). There was a definite black stripe extending along the long axis of the body. The stripe connected two small black points (Pl. XIII, E, 1, 2, 4, 5, 6, 8, 9). Some of these bodies, after they turned around the long axis, showed a cap-shaped structure adherent to the whole length of the body (Pl. XIII, E, 6). The external edge of the additional structure was either semi-circular or showed an angle. The black stripe was not visible if the body was viewed from the cap-shaped side. In the transitional zone of a mosaic leaf which contained chloroplasts with the hyaline motile bodies there were peculiar transparent spheres attached to the chloroplasts. Careful examination of the material revealed different stages of the dissolution of the chloroplasts and formation of the transparent spheres. The intermediate phases of this process are illustrated in Plate XIII, C and D. In the earliest stage, a blister-like protrusion begins to appear along one side of the chloroplast (Pl. XIII, C, 4). The moving hyaline bodies, two or more in number, at that stage of the formation of the protrusion, are visible only in the chloroplast. In some other chloroplasts the blister-like spheres were about half the size of the plastid (Pl. XIII, C, 5). When the spheres are larger than the plastid, the actively motile bodies are no longer seen in the chloroplasts, but they move about within the spheres. Finally the chloroplast disappears entirely and the sphere reaches a size of about 10μ in diameter. Numerous spheres may occupy the entire cell, each sphere containing actively moving hyaline bodies. Successful perma-

nent preparations of the spheres and late stages of the dissolution of the chloroplasts in the cells of the chlorotic region were obtained by fixing the material with one per cent picric acid and staining with aniline blue. A camera lucida drawing of such a preparation is represented in Plate XIII, B. The disintegrating chloroplasts appear brown; the spheres are almost transparent and slightly greenish. The hyaline bodies inside of the spheres are not fixed well enough by this method to be distinct. By treating the leaves containing rather small chlorotic and green areas by the above described method, it is easy to obtain excellent preparations showing the difference in the chloroplasts of the diseased and healthy tissues. The chloroplasts of the green area stain bright green (yellow after picric and blue after aniline blue), while the chlorotic region contains the brownish remains of the chloroplasts and the transparent spheres. The brilliant green spots on the slide are easily distinguishable from the brownish-yellow spots.

PHYSICAL AND CHEMICAL PROPERTIES OF THE BODIES ASSOCIATED WITH THE DESTRUCTION OF CHLOROPLASTS

For the study of the physical and chemical properties of the chloroplasts and the spheres it seemed desirable to isolate both of them from the other cell contents. This was accomplished by tearing the epidermis and the palisade parenchyma with needles under a dissecting binocular and mounting the resulting mass of chloroplasts and spheres in distilled water. By regulating the amount of water carefully, it was possible to observe the chloroplasts and the spheres on the same preparation for several days. To make the spheres more distinct, some dilute solutions of stain were applied to the mounted material. Of the typical vital stains, only thionin stained the spheres slightly. A dilute solution of Giemsa stain applied in vivo stained the spheres light violet. Dilute water solution of gentian violet proved to be very satisfactory. The chloroplasts appeared dark purple and the spheres lavender, while motile bodies did not take the stain.

Mounts of the spheres and chloroplasts from the transitional zone between chlorotic and green areas of a diseased leaf, which were prepared and stained in vivo as above described, were photographed with a moving picture camera. In Plate XIV, A are represented enlarged prints from the positives. The majority of the chloroplasts exhibit the phenomenon of dissolution of the mass of the body. Various transitional stages of this process can be found in the accompanying illustrations. The white ellipsoidal bodies on the print are the chloroplasts. A considerable number of them are healthy, while others are in different stages of the dissolution. There also are found on the print numerous complete spheres without any of the mass of the chloroplast adhering to them. The movement of the

hyaline bodies inside of the spheres is evident when the film is projected on the screen. A single print, however, does not show the bodies.

The spheres are very regularly spherical in shape and vary in size from 8–10 μ in diameter. In living condition they seem to be homogeneous in structure, transparent and slightly reflecting. There usually are two or three moving bodies inside of those spheres to which a considerable amount of the mass of the chloroplast still adheres. When the chloroplasts have completely disappeared, there are more moving bodies inside each of the spheres, the average being from 5 to 8 and occasionally as many as 15.

In iodine-potassium iodide solution the spheres become yellowish-brown, while the moving bodies inside of them become a deeper brown. This reagent does not stop the movement of the hyaline bodies. With the addition of concentrated H_2SO_4 to the material mounted in I_2KI, some of the spheres collapse; others remain spherical or shrink only slightly and retain their brownish color. If the reagent is applied to the spheres in the tissues of tomato, the hydrocellulose reaction is very evident in the neighboring cells, but the spheres give no indication of the cellulose reaction. The reactions for fats are negative. The usual protein reactions are very indefinite, usually negative.

The spheres and the moving hyaline bodies have a peculiar reaction towards alcohol. The grades of alcohol below 85 per cent do not have a distinct effect, but 85 per cent alcohol will stop the movement of the larger hyaline bodies while the smaller ones still move, and in 95 per cent alcohol the movement of all hyaline bodies ceases. The spheres partly collapse and shrink, but they are not dissolved even in absolute alcohol. A gradual addition of acetone to material in water does not affect the spheres nor the moving hyaline bodies. When almost all the water is replaced by acetone, the spheres become especially distinct while the bodies still continue moving.

The spheres are not dissolved in 10 per cent hydrochloric acid, but a weak solution of KOH will dissolve them. The movement of the hyaline bodies is entirely stopped by the action of alkaline solutions.

It is interesting to note here that even 10 per cent KOH does not have any appreciable effect on the chloroplasts. If the chloroplasts have swollen after having been mounted in water and are then subjected to the action of 10 per cent KOH, they react in the same way as non-swollen chloroplasts. The reaction is entirely different from the reaction of the spheres to KOH. This fact indicates that the substances present in the spheres are entirely different from those in the swollen chloroplasts.

A technique commonly used in the study of the animal virus diseases was tried on the diseased tomato tissue for a comparison of the pathological cell inclusions of plants and animals. Chloroplasts and spheres from the diseased leaf were mounted in a drop of water and exposed to osmic acid

vapor for about 5 to 10 minutes. The preparation was then dried very quickly by heating in the flame of an alcohol lamp. It was then stained with basic fuchsin according to Löffler [described by Giemsa (7)], or by the usual Giemsa method without the addition of Na_2CO_3. The spheres are rather resistant to stains and usually color along the periphery and in the meshwork inside, which is very distinct in fixed material. A deeper color of the whole structure is obtained by staining for a longer time, or by using gentian violet, for which the spheres have a special affinity. After staining with Löffler's stain the spheres appear rose-colored, the chloroplasts red, and the bodies inside of the spheres become dark, almost black-red. In Plate XV, B is represented a smear preparation of the spheres stained by Löffler's method. The spheres show a definite structure inside, which consists of granules and threads. The dark body next to the sphere is a chloroplast and the three distinct dark spots inside of the sphere are the "hyaline bodies," while the whole sphere shows peculiar lighter stained threads and granules which were not seen in the living material. It is quite evident that the granules and threads are found inside of the sphere and are not merely the shrinking of the surface of the sphere, the outlines of which are perfectly regular. In Plate XV, C is shown a later stage of the dissolution of the chloroplasts, the mass of which is somewhat elongated and spread out on the surface of the sphere. A distinct dark body is seen inside the sphere. Here also, besides the body inside of the sphere, it is possible to observe the peculiar structure. A similar condition is shown in the preparations stained with Giemsa stain (Pl. XV, D); in the upper corner to the right a healthy chloroplast is found; the two other plastids are shown in various stages of dissolution.

The chloroplasts with the adherent spheres at first suggest the well-known phenomenon of swelling which was studied long ago by Mohl (25), Sachs (31), Hofmeister (11) and others. Liebaldt (20) described sudden swelling of the chloroplasts of certain plants after they had been brought in contact with water. She found two types of swelling in the chloroplasts. The first and most common type consisted in the general increase of the size of the plastids and the appearance of vacuoles inside the stroma. The second type consisted in the formation of vesicles adherent to the chloroplasts. The conditions which determined this latter type of swelling were rather indefinite; sometimes chloroplasts of a certain species exhibited this kind of swelling, while in other cases they did not. It is quite possible that the first type was a case of real swelling, while the second may have been an entirely different phenomenon.

Zirkle (35) studied the swelling of the chloroplasts in fern prothallia and in different higher plants. His conclusions are that the phenomenon is exhibited not by the stroma of the plastid but by the substance within the

central vacuole. The stroma either remains adhering to the vacuole on one side, as in fern prothallia, or is ruptured in numerous places and distributed over the surface film of the vacuolar contents. Attempts to stain the contents of these vacuoles *in vivo* were not successful.

I observed in the same mount of diseased tomato tissue normal chloroplasts, chloroplasts with attached spheres, and transparent spheres without any amount of the stroma of the chloroplasts adhering to them. This indicates that the phenomenon is not merely a swelling of the chloroplasts as a result of contact with water. There seemed to be some factor which caused essential changes in some of the chloroplasts, as a result of which the swelling occurred. This factor was absent in the normal chloroplasts.

Before offering an hypothesis to explain the dissolution of the mass of the chloroplasts, certain physico-chemical properties of chloroplasts should be reviewed. The general substance of the chloroplast is a protein, according to most authors. As emphasized by Zirkle (35), the typical protein reactions are positive, but "not as distinct, as could be wished." Proteolytic enzymes, however, will digest chloroplasts. According to Lepeschkin (18, 19), chloroplasts and protoplasm are built in a similar way, and constitute colloidal systems. The disperse phase in the active cell is an organic fluid which consists of a loose chemical combination of proteids and lipoids. These chemical combinations imbibe water in great amount until a certain limit is reached, after which the system becomes saturated and vacuoles appear. The dispersion medium and disperse phases of chloroplasts and protoplasm are, however, chemically different.

The condition of aggregation and the consistency of the chloroplasts are changeable; sometimes they are fluid, sometimes the viscosity is so great that they become gelatinous. The method of isolation of chloroplasts from the cells, which was practised by Lubimenko[3] to a great extent, indicates that normal chloroplasts are rather solid or semisolid. This method consists in soaking leaf fragments in water for several weeks until the leaf tissue decays, when the chloroplasts fall to the bottom of the dish. The microscopic studies of the chloroplasts treated in this way showed that such a treatment did not affect the plastids very seriously. Zirkle (35) also made some observations on the chloroplasts. By teasing the chloroplasts with needles he noticed that the fragments maintained their shape, from which he concluded that the chloroplasts consist of a gel rather than a sol.

From the point of view of the colloidal-chemical theory of the structure of chloroplasts, the formation of vacuoles in chloroplasts is the resultant of syneresis following the phenomenon of typical swelling or solution of water in the disperse phase. The formation of spheres in diseased tomato mate-

[3] The writer was unable to locate this paper by Lubimenko. The results given are from personal information obtained from the author.

rial, on the other hand, is apparently just the reverse: it is probably a solution of the colloid in the solvent.

Fischer (6), in his excellent book, says: "The phenomena of swelling (hydration) and of 'solution' in protein gels, while frequently associated, are essentially different. Swelling is best understood as a change whereby the protein enters into physico-chemical combination with more of the solvent (water), as a change in the direction of greater solubility of the solvent in the protein; 'solution' is best conceived of as a change in the direction of greater solubility (an increased degree of dispersion) of the colloid in the solvent."

The following hypothesis seems to explain the changes in the chloroplasts of the diseased tomato plant. Observations made when teasing apart the chloroplasts in tomato plants indicate that they are normally rather solid. The first indications of a pathological condition are the appearance of hyaline bodies inside the chloroplasts and later the movement of these bodies. Previous to this movement, a liquefaction of the stroma must have taken place which may possibly be the result of the action of an organism secreting a proteolytic enzyme. This proteolytic enzyme penetrates into the chloroplast and causes the digestion of the proteins. As a result, the osmotic pressure increases inside, and water is taken in from the surrounding medium. Finally, all of the body of the chloroplast goes into solution and the sphere appears. Therefore the sphere may well represent a "membrane" resulting from the digested chloroplast in which the digestion products and water are retained, the whole being surrounded by a surface film at the interface with water, and containing moving bodies. The supposed organisms have apparently quite unusual properties, different from the properties of the generally accepted conception of an organism. Whether the "organisms" are ultramicroscopic or whether the hyaline bodies are the "organisms" is impossible to say at the present stage of our knowledge.

The spheres are found in large numbers in the very chlorotic regions of mosaic tomato leaves. The greater part of the spheres in these areas are without any trace of the chloroplast. In the non-chlorotic parts of mosaic leaves, the spheres, if present, usually have a considerable amount of the chloroplast mass adhering to them. But as a rule they are not so abundant in these regions as in the chlorotic areas. As to the presence or absence of the spheres in absolutely healthy plants, no definite results have been obtained. Special attempts to grow absolutely healthy plants were not made. On apparently healthy plants taken from the field, several hundreds of observations were made. In the majority of cases the chloroplasts were normal and did not have any spheres. Sometimes one or several spheres were found in the whole mount. This condition could not be compared with the abundance of spheres in the mounts from definitely diseased material.

In order to find out whether the spheres are found in insects, smear preparations of the contents of the intestines were made from white fly and mealy bug which were feeding on mosaic tomato. The methods of fixation and staining were the same as those used for the spheres in the plants. Photomicrographs (Pl. XV, E, F) show spheres from the insects to be indistinguishable from those found in the plant.[4]

"MOSAIC BODIES" OF TOMATO LEAF COMPARED WITH SOME OF THE INCLUSIONS FOUND IN CELLS OF ANIMALS AFFECTED WITH VIRUS DISEASES

Of the different pathological cell inclusions which are found in various animal virus diseases, the polyhedral bodies of lepidopterous larvae deserve special attention. They were studied by Prowazek (27, 28, 29), Glaser and Chapman (8), Komarek and Breindl (13), and others. The physico-chemical properties of these structures are well known. The polyhedral bodies from *Lymmonacha*, according to Komarek and Breindl (13), are 5–10 μ in diameter and tetrahedral in shape with somewhat rounded angles. In the young stage they are spherical. Their structure is homogenous. They are transparent and reflecting, and contain rapidly moving bodies. They give positive protein reaction, are not soluble in alcohol, chloroform, ether, or benzol, are comparatively resistant to weak acids, and are very sensitive towards alkali. When stained with Löffler's fuchsin red, they show definite structure; after Giemsa stain they appear blue, the peripheral part always being deeper in color.

The writer did not have opportunity to see the preparations of the polyhedral bodies, but, from the drawings as represented in the above mentioned papers, they resemble somewhat the spheres from diseased tomato. The size of the two structures is the same, and some of the physical and chemical properties, as well as their staining reactions, correspond very closely. Nevertheless, very essential differences should be emphasized. In the spheres, the positive protein reaction was absent. According to Prowazek (28), however, the protein reaction in the polyhedral bodies is not always positive. A very interesting analogy could be found between the polyhedral bodies and the spheres. In the diseased nuclei of the insects the chromatin material flows together and the polyhedral bodies arise as very minute structures. They gradually increase in size, and the chromatin is used up during the synthetic process. As the polyhedral bodies grow, they become more and more refractive, and stain with difficulty. Finally, the whole nucleus is transformed into a mass of polyhedral bodies. Similarly, in the

[4] The difference in size of the photomicrographs is due to the difference in magnification.

chloroplasts of the diseased leaves the spheres are formed as a result of breaking down of the body of the chloroplast.

As to whether the polyhedral bodies are the degeneration products of the host, or a certain stage in the development of a parasitic organism, opinions differ. Knoche [according to Komarek and Breindl (13)] finds that they are a resting stage of an unknown organism. Glaser and Chapman (8) and Prowazek (27, 28, 29) think that they are products of the host. Komarek and Breindl (13) concluded that the polyhedrons are not real cysts or spores of an organism but are specific products of reaction of the nuclear substance, which could be compared with the formation of certain galls. But at the same time, the polyhedrons are the final stage in the life history of a Chlamydozoon, representing a resting stage of the latter.

In the cells of tomato leaf affected with mosaic, which were devoid of the normal components, there were hyaline bodies which moved with great rapidity. Some of these bodies were almost identical with the bodies found in the spheres. These bodies were very sensitive to alkali and resistant to many other chemicals. In addition to the bodies identical with these found in the spheres, there were also moving bodies of slightly different type. These were mostly much larger, between 2 and 5 μ, more irregular in shape and more sensitive to some chemicals. They reminded one of pictures of the hereditarily transmitted microorganisms of the class of Rickettsia, which were found by Cowdry (2) in some insects, and apparently unassociated with any disease. An interesting phenomenon was described by Cowdry (3) in the heartwater disease of cattle, which is due to virus transmitted by the bont tick. He found that in the adults, larvae, and nymphs of *Amblyomma hebraeum*, side by side with *Rickettsia ruminantium* which has been proved to be the etiological agent, there were other organisms apparently unassociated with the disease. They are larger than most of the Rickettsiae, decidedly pleomorphic, gram-negative and intracellular. In some cases the entire microorganism appeared brightly luminous, while in others the luminous material was confined to a thin marginal layer. The organisms varied from spherules to straight and curved rods and filaments. Similar microorganisms transmitted hereditarily were present in at least sixteen species of ticks.

Hertig and Wolbach (10) stated that minute microorganisms morphologically and tinctorially similar to the pathogenic Rickettsiae are widely distributed in arthropods, without relation to their feeding habits.

As mentioned above, at the present stage of our knowledge we cannot say whether the hyaline bodies are organisms or not. In some of their properties they correspond with our conception of an organism; in others they do not. The wide distribution of various kinds of hyaline bodies in plants is not negative evidence of the relation of some of these structures

to the mosaic disease. The literature we have reviewed on the organisms of similar structure in insects suggests that it is quite possible they are parasitic as well as symbiotic. This would explain the occurrence of symbiotic Rickettsiae in insects which are feeding exclusively on plant juices.

A third type of moving body was found in abundance in the hair cells. These bodies are very similar in structure to the hyaline bodies found in the spheres, except that they are not green reflecting. In all other properties they correspond exactly with the hyaline bodies in the spheres. Plate XIII, F is a camera lucida drawing of a living hair cell containing the bodies. They occupy the central part of the cell. There is very intensive, typical movement. The components of the cell nucleus, cytoplasm and leucoplasts are shown to be normally developed. Plate XIII, G illustrates a peculiar transitional stage of the bodies from the motile into resting condition. After a period of observation, the whole mass of the bodies became suddenly surrounded by a halo of clear cytoplasm. The clump was distinctly heterogeneous, composed of the bodies and a matrix. All motility stopped as soon as the bodies were completely surrounded. Similarly, a tendency of the Rickettsiae to aggregate into clumps in the tissues and in the cells of mammals and insects has been described by Cowdry (3).

In Plate XIII, H is represented a cell which has been plasmolyzed by a sugar solution. Here the cytoplasm plasmolyzes quite independently of the bodies. The latter collapse together and surround themselves by a sort of membrane. In the fixed and stained material the bodies do not retain their typical shape. This probably is the reason that very little attention has been paid to them. Possibly, however, Iwanowski (12) had in mind this kind of "organism" when he mentioned the occurrence of bacteria in the cells of tobacco leaves affected with mosaic. And the granular substance reported by several authors quite probably is a mass of fixed moving bodies.

In order to be sure of the effect of fixation on these bodies, the whole process of killing and staining was performed on the slide under the microscope, so that each transformation of the moving bodies was observed. In Plate XV, H is represented a hair cell from a mosaic tomato leaf fixed with Zenker's fluid and stained with gentian violet and eosin. The cytoplasm in this cell occupies the center; it is plasmolyzed and stained pink, and the nucleus is violet. Between the cytoplasm and the cell wall the bodies are found in abundance. They are brilliant violet-blue. Figure G shows the bodies localized only in one part of the cell, the cytoplasm having been destroyed in this part. The same combination of stains already described shows that the area occupied by the bodies is colorless, and the bodies stain a deep blue-violet, while the rest of the cytoplasm of the hair cell stains pink. The effect of the bodies on the stain is shown in Plate XV, I. The cell to the left is more or less healthy, it stains pink, and does not show any abnor-

mal inclusions. The cell to the right stains blue-violet on account of the large number of bodies inside it.

Strongyloplasma iwanowskii was the name given by Palm (26) to the supposed causal organism of mosaic disease in tobacco. He found some minute granules in the diseased cells, which he believes correspond to the organisms described as bacteria by Iwanowski. Some larger granules were recorded also. Apparently the granules of two different sizes noted by Palm correspond to the two types of bodies found in tomato mosaic.

The question whether the moving bodies are real organisms or the by-product of the activity of an ultramicroscopic organism, as has been mentioned, is very difficult to answer. One thing, however, is certain. An action of an organism is involved in the processes described and this organism possesses unusual properties. Some of these properties correspond very closely with certain properties of Chlamydozoa, Strongyloplasma (21), and Rickettsiae, which are already accepted by the protozoologists as being real organisms. Therefore it seems quite logical to conclude that an organism of the Chlamydozoon type is involved in the mosaic disease of tomato, the term Chlamydozoa being used here for a collective conception.

The fact that the destruction of the chloroplasts in tomato mosaic is associated with a Chlamydozoon type of organism is of great importance, because it provides a link between this type of disease and the animal virus diseases, the greater part of which, it has been demonstrated, are caused by an organism of this kind.

Kunkel (15, 16) thinks that the intracellular bodies found in plants with mosaic disease call to mind the intracellular bodies associated with certain of the virus diseases of animals. But in the animal diseases there are always present some minute inclusions of the chlamydozoa type inside of the bigger intracellular bodies, as, for instance, in the Negri bodies. The inclusions are really considered as the organisms which cause the disease. Such inclusions it seems are absent from the intracellular bodies of the mosaic-affected plants. Magrou (22) finds, on the other hand, that some of the pictures of Kunkel and the descriptions of Palm are very similar to a Rickettsia type of organism.

Among the other inclusions in cells of tomato affected with mosaic may be mentioned intracellular bodies of Iwanowski (12), Kunkel (15, 16, 17), Palm (26), McKinney, Eckerson and Webb (23), Goldstein (9), Smith (32), Cook (1), Rawlins and Johnson (30), and others. These bodies are quite definitely associated with the disease and are always found in the cells of mosaic tomato in which there are many crystals. The physical and chemical properties of these intracellular bodies correspond exactly with the properties of the living protoplasm. They are immediately killed with all of the reagents which ordinarily kill the cytoplasm of most cells, and do not

correspond with the peculiar reaction of the virus to certain chemicals like alcohol, acetone, acids and so on. Possibly they represent one of the stages of the life history of an organism.

The presence of crystals is very typical of cells in which intracellular bodies are found. Very commonly it is possible to see in one and the same cell intracellular bodies and the two types of moving hyaline bodies.

As to the "striate material" described by several authors, such a formation has never been observed in living cells. In the fixed material it is a quite constant inclusion of the cells which contain the intracellular bodies. By fixing the material under the microscope it was possible to observe the transformation of the crystals into striate material. Especially effective in this respect is picric acid. After half an hour in one per cent water solution, all of the large crystals were transformed into striate material.

SUMMARY

1. In the decidedly chlorotic region of a young leaf of tomato affected with severe mosaic disease, all of the cells are nearly isodiametric. There is no differentiation of tissue into palisade and spongy parenchyma. In a majority of the cells the chloroplasts are absent or present in small numbers. A granular substance often occupies the cells in which the normal components are lacking.

2. The chloroplasts are destroyed through the dissolution of the proteins of the stroma. This fact is easily demonstrated by direct observation of the living material and by microchemical tests. The same is confirmed by study of the material fixed under the microscope, or fixed and stained by usual cytological methods.

3. The normal chloroplasts are rather solid. The first indication of a pathological condition is the appearance of rapidly moving hyaline bodies within the chloroplasts. The movement of these bodies is possible only after liquification of the stroma of the chloroplasts has taken place. Therefore it is assumed that a proteolytic enzyme, possibly secreted by an organism, is present. An increased osmotic concentration results inside of the chloroplast, and water is taken in from the surrounding medium. Finally, the entire body of the chloroplast goes into solution and, if sufficient water is present in the surrounding medium, spherical transparent vesicles result.

4. The sphere represents a "membrane" resulting from the digested chloroplast in which the digestion products and water are retained, the whole being surrounded by a surface film at the interface. The spheres give negative protein reactions, and are not soluble in alcohol, acetone, or acids. They are soluble in weak alkali. They can be fixed and stained by the methods used in the study of the inclusion of the animal virus diseases.

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EXPLANATION OF PLATES

PLATE XII

Photomicrographs. Zeiss 8, Apert. 65, Comp. Oc. 8.

A. Section through chlorotic region of a mosaic tomato leaf about three weeks old.
B. Section through a green area of the same leaf.
C. Section through the transitional zone between the two areas.
All three photographs were from the same section.

PLATE XIII

Camera lucida drawings. Zeiss Apochr. Oil Imm. 3, Apert 1. 30, Comp. Oc. 12.
Reduced four times in reproduction.

A. Surface view of a transitional zone of a mosaic tomato leaf, focused through the epidermis.
B. Surface view of parenchyma cells from a chlorotic region of a mosaic tomato leaf. The transparent spheres result from the destroyed chloroplasts.
C and D. Stages in the destruction of chloroplasts and formation of spheres.
C. 1.—Structure of a healthy chloroplast.
2.—Early stage of disintegration of the chloroplasts showing stationary hyaline bodies surrounded by a halo.
3.—Later stage of disintegration at which the hyaline bodies become motile.
4.—A sphere appearing on one side of the chloroplast.
5.—The sphere increased in size.
D. 1.—The motile bodies in the sphere.
2–5.—Gradual stages of the complete dissolution of the mass of the chloroplast.

E. The hyaline bodies from the spheres. (Zeiss Apochr. 3, Apert. 1. 30, Comp. Oc. 18.) Some of the characteristic shapes of the bodies. From the front view the bodies appear more or less crystal-like with a definite black stripe connecting two black points. From the side view the body has a cap-shaped addition.

F. Living hair cell from a mosaic tomato leaf. (Leitz 6, Oc. 10.) The development of the bodies in the cell vacuole. The bodies have a very distinct and rapidly rotating movement and are highly luminous.

G. The same cell as in F. The bodies suddenly clump together, lose their motility, and assume a membrane.

H. Hair cell, similar to one described in F, after plasmolysis. The cytoplasm plasmolyzes independently from the mass of the bodies, which in turn clump together.

PLATE XIV

Motion photomicrograph of living chloroplasts and spheres.

A. A print from the positive. The white bodies are the chloroplasts. The transparent vesicles are the spheres. Different stages in the dissolution of the bodies of the chloroplasts are shown in the print.

PLATE XV

Photomicrographs. Zeiss Apochr. 3, Apert. 1. 30, Comp. Oc. 12.

A. Chloroplast stained with Löffler's stain.

B. Chloroplast in the stage of destruction. The dark body below is the chloroplast. The structure on top of it is the sphere. Definite granules and threads are visible inside of the sphere.

C. Later stage in the dissolution of the chloroplast body.

D. Series of stages similar to A, B and C, after Giemsa stain.

E and F. Spheres from the intestines of insects feeding on diseased tomato.

G. A hair cell from the diseased material fixed with Zenker and stained with gentian violet and eosin. The cytoplasm appears brilliant pink, the nucleus purple, the bodies, which occupy the space between the cytoplasm and the cell wall, are brilliant blue.

H. A hair cell from diseased material treated as in G. The mass of the bodies is localized in a certain portion of a hair cell. The cytoplasm of the unaffected portion of the cell is pink, the bodies are brilliant blue. The portion of the cell around the mass of the bodies is white, the cytoplasm apparently being destroyed there.

I. Two cells of the same hair, treated as in G. One to the left is healthy, the cytoplasm is homogenous in structure, and is stained pink. In the cell to the right there is an enormous number of bodies, so that the whole cell is stained blue.

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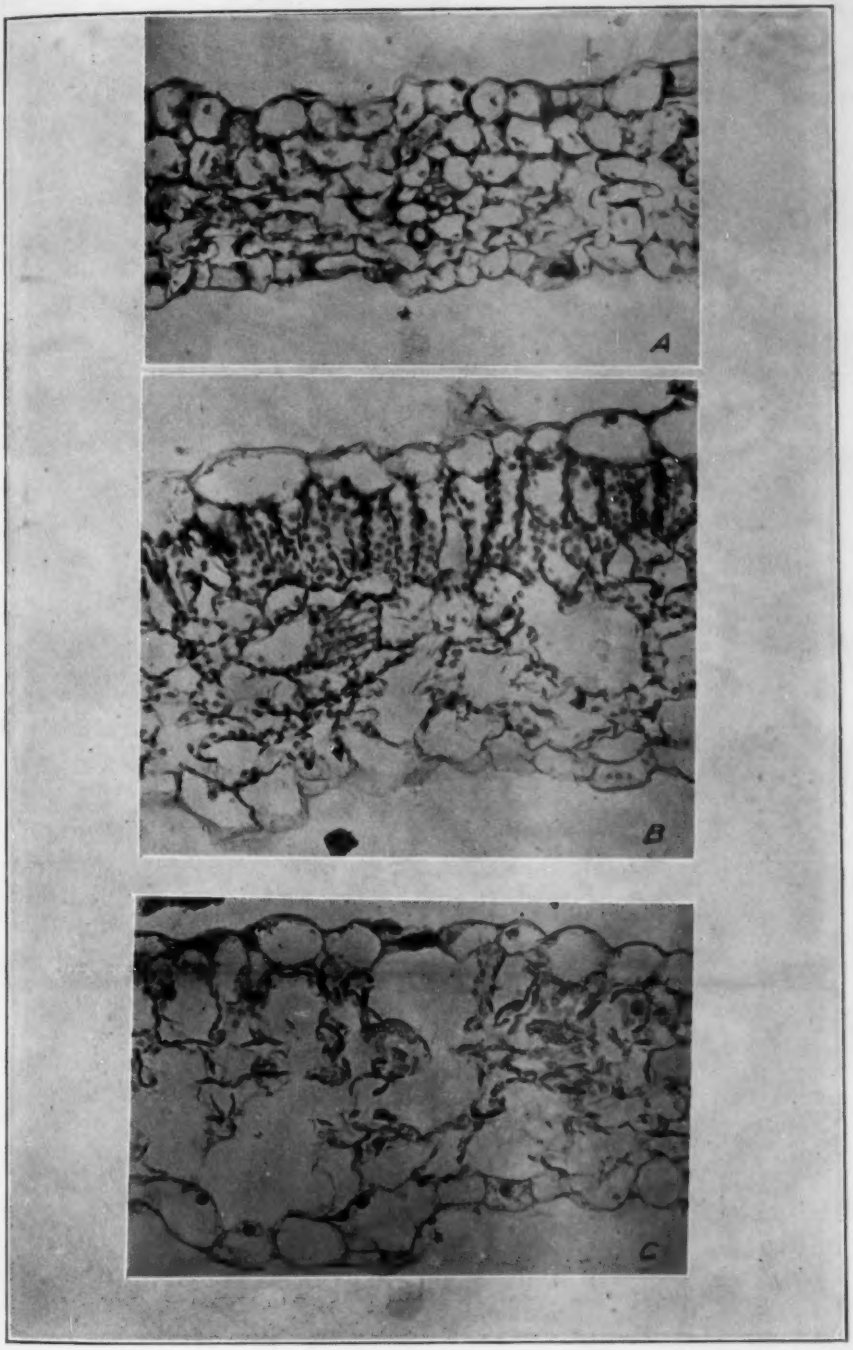
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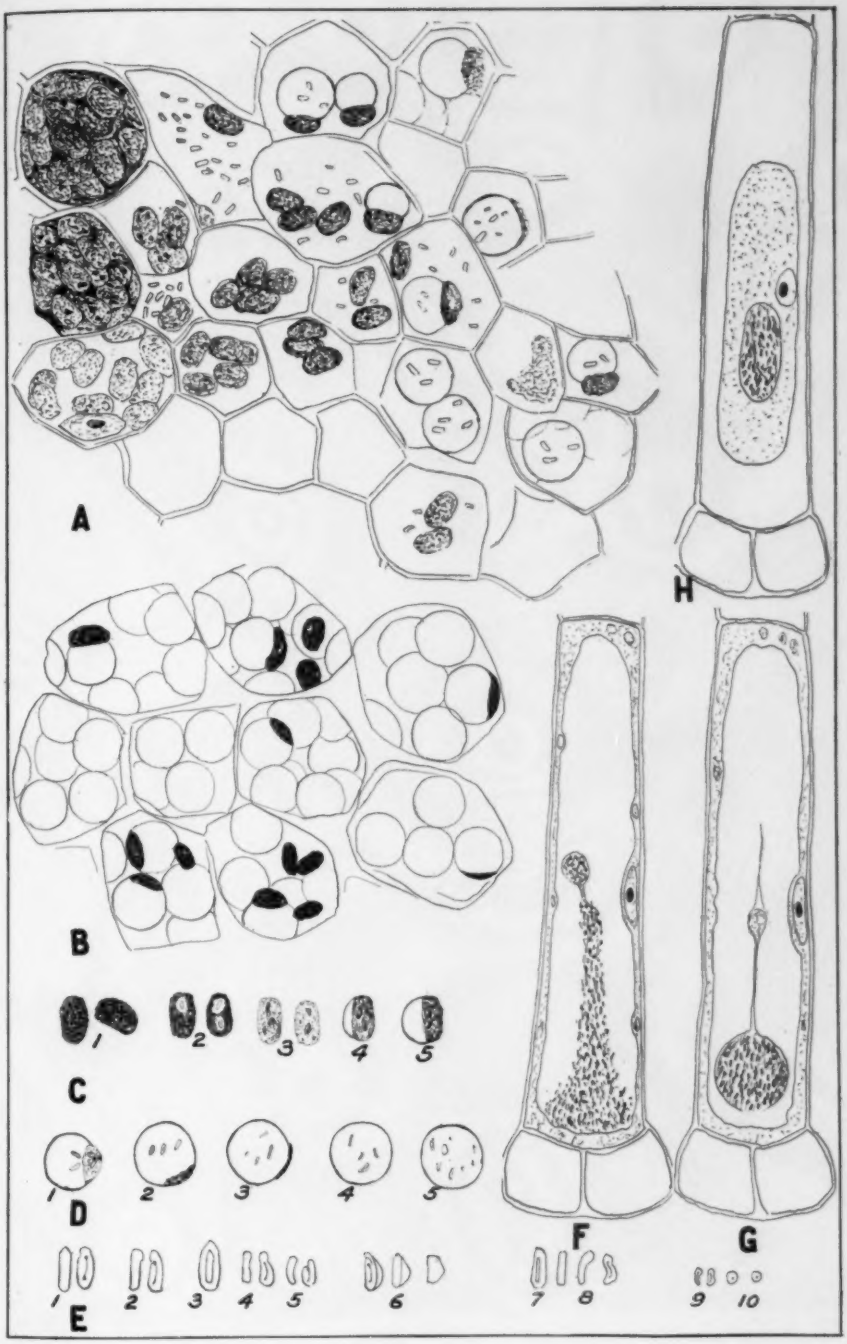
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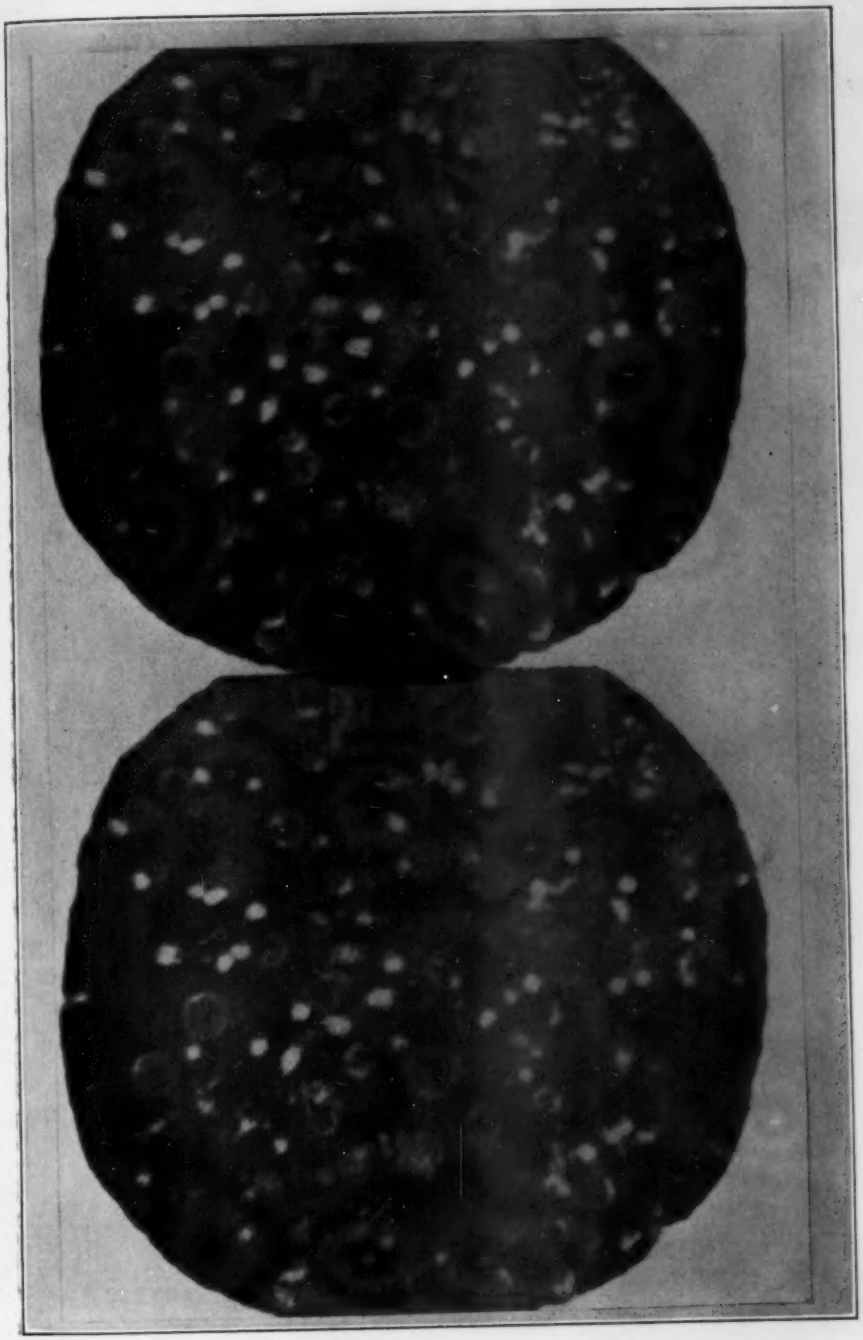

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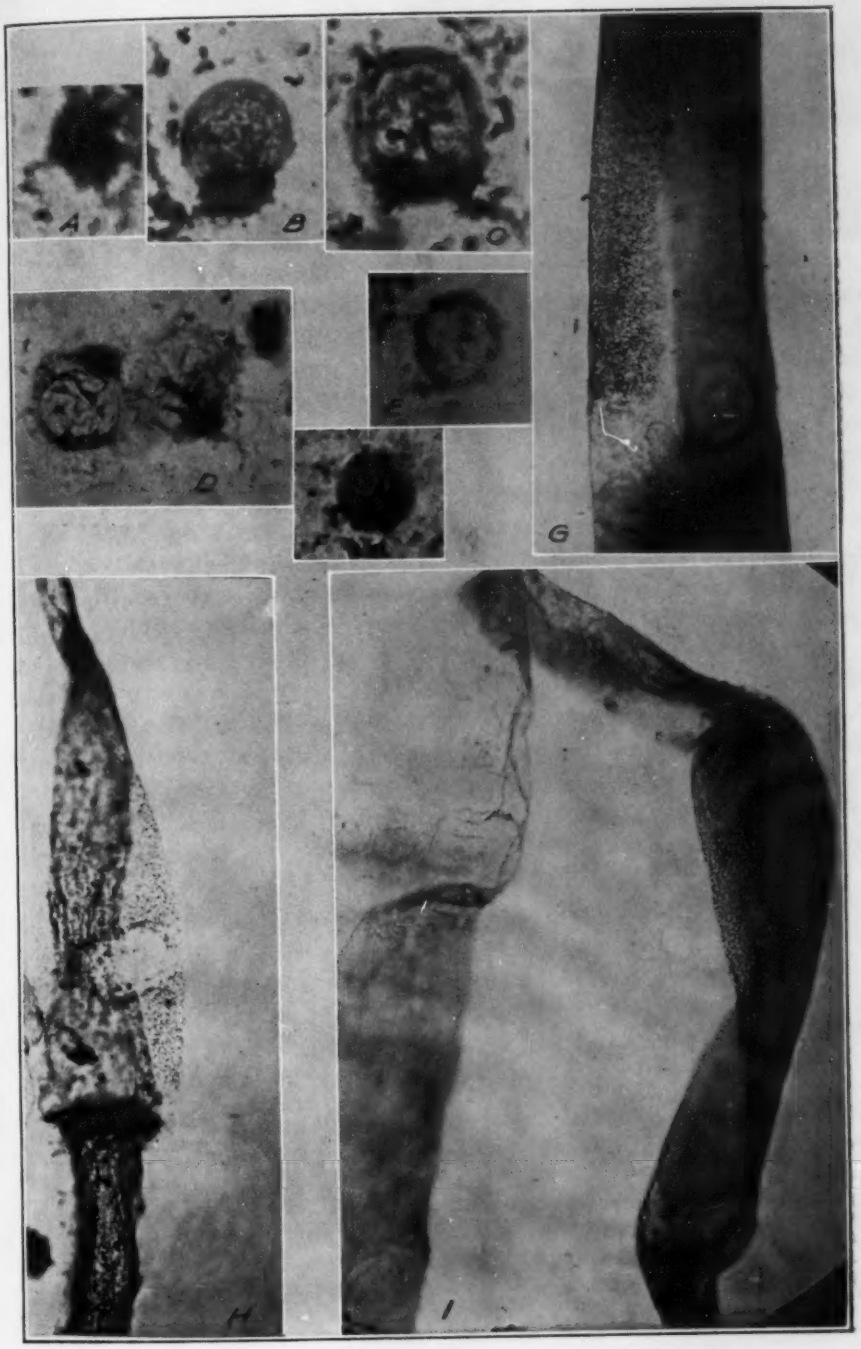
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SPECIES OF ASCOCHYTA PARASITIC ON THE PEA[1]

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INTRODUCTION

Among the several diseases of the pea (*Pisum sativum* L.) which have demanded study as an outcome of the Wisconsin pea disease survey (9) is the well-known leafspot and podspot attributed to the fungus *Ascochyta pisi* Lib., the imperfect stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone. Attention was drawn to this disease in 1924, when, in fields from which foliage spotting was absent, a footrot was found which yielded cultures of an *Ascochyta*. These cultures produced very small, chiefly nonseptate pycnospores and were regarded at the time as a Phoma-like fungus (9) but later (5) as a species of *Ascochyta*[2] probably distinct from *A. pisi*.

In 1925, when an attempt was made to compare this fungus with *Ascochyta pisi*, two distinctly different and markedly constant types of *Ascochyta* leafspot were observed in Wisconsin fields; one characteristically regular and light brown in color, the other irregular and dark brown. Measurements of pycnospores revealed the presence of three distinct fungi associated with the two types of spots. The light-brown spots contain only a typical *Ascochyta* with spores larger than those of the footrot fungus. The dark spots, on the other hand, contain two different fungi as indicated by spore characters. The one usually present is a typical *Ascochyta* with spores somewhat larger than those found in light-brown spots. The other fungus, found less frequently, is apparently identical with the footrot fungus.

Likewise, isolations from these two classes of leafspots have yielded three different types of cultures. Cultures from the light spots fall readily into a group by themselves. Those from the dark spots are all similar in macroscopic appearance, but microscopically they are of two types with respect to spore characters. The predominant type is a typical *Ascochyta*, but less frequently the footrot fungus is found.

[1] Contribution from pea disease investigations conducted at Madison, Wisconsin, supported jointly by the Wisconsin Pea Packers' Association, The Wisconsin Agricultural Experiment Station, and the Office of Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture.

The writers wish to express their gratitude to Dr. F. R. Jones for his advice and assistance, both in the progress of this work and in preparation of the manuscript.

[2] This is probably identical with both Haenseler's (6) *Phoma* sp. and his "sterile fungus."

After Jones (10) had emphasized the importance of *Ascochyta* infection of pea seed for canning-crop production, numerous isolations of *Ascochyta* from seed were made at Madison, and the cultures thus obtained were found to fall readily into the three classes already recognized.

In this paper these three types of *Ascochyta*, isolated from two classes of leafspots, from footrot, and from diseased seed, are compared. For convenience, spots of the light-brown type, together with the fungus which they bear and cultures of the type isolated from such spots, are referred to as "light"; spots of the dark-brown, zonate class with the corresponding fungus and cultures are designated "dark"; and the footrot fungus and cultures are termed "micro." A partial report of this work has already been published (14).

SYMPTOMS ON AERIAL PARTS

The two classes of spotting caused by *Ascochyta* on pea leaves, stems, and pods, readily distinguished by observation of well developed symptoms, are as follows:

"Light"

The light type of spotting (Plate XVI, A–C) is characterized by a circular, tan spot, sometimes whitish at the center, with a distinct dark-brown margin, and with amber to brown pycnidia fairly conspicuous towards the center. Spots on leaves are circular or oval and from 2 to 10 mm. in diameter. On stems, petioles, and pedicels they are deep and oval or elongate, and may almost sever the affected part. On pods, deep, circular, tan pits bearing numerous pycnidia and surrounded by a prominent dark margin frequently penetrate and discolor the peas within. Late infection of older pods may lead to the production of pycnidia without the formation of definite spots.

"Dark"

At a glance this type of spotting differs from the light in its dark, irregularly dendritic or zonate character. Minute dark-brown points first appear, which may enlarge dendritically (Plate XVI, D), retaining a dark-brown, chocolate, or purplish-brown shade. On dried specimens these small spots often appear elevated. Becoming larger, they may assume a circular outline and a zonate aspect (Plate XVI, E–G). Zonate spots are usually lighter than the smaller, homogenous ones, but they do not approach the tan color of light spots. They are generally darkest at the center, sometimes pale-gray-brown in the peripheral zones. Frequently they are surrounded by a zone of affected tissue which is still green. Where spots are numerous usually but few attain a size as large as 8 mm. in diameter. On stems,

petioles, and pods this dark spotting is commonly more superficial and spreading than the light. Stem lesions occur especially near the ground and at nodes, and may spread over considerable areas, causing a dark-brown to purplish-brown discoloration of the cortical tissue.

Often abundant infection will result in only minute brown flecks and streaks. On pods such flecks occur frequently, but well developed spots are less common than with the light type. When they do occur, dark spots on pods are less abruptly sunken, darker, and usually purplish-brown to gray-brown in color, and are often zonate, as on leaves. Late infections may, however, lead to fruiting on the pod without forming any spots, a condition macroscopically indistinguishable from the similar development of the light fungus.

Pycnidia are produced erratically in spots of the dark type. Many spots, apparently well developed, contain none; and, when present, the pycnidia are inconspicuous against their dark background, and are scattered near the periphery rather than clustered at the center of the spot. Occasionally, however, they are abundant.

THE FUNGI

Morphological Differences

For a comparison of the fungi associated with these two types of leaf-spot, exsiccatae from various parts of the United States and Europe were examined. From these sources and from freshly collected specimens, 20 samples of each type of spot were selected, in addition to four samples of the micro fungus from freshly collected dark-type spots. Twenty-five spores from each sample were measured under the oil immersion lens.

Considerable variation was observed between the various samples of each class, spores from podspots being more variable than those from leafspots; but the spores from light spots are clearly shorter and somewhat narrower than the spores from the dark ones, excepting only that the micro spores from occasional dark spots are smaller still. Mean spore lengths of light samples are nearly all less than 13 μ, while those of the dark are, with two exceptions, greater than 13.5 μ (Fig. 1). Differences in breadth are evident but less striking.

Visual examination of the spores reveals certain differences not brought out by measurements. The dark spores appear plumper and more constricted than the light, and fresh spores of the former contain more conspicuous oil droplets. Counts of the spores of the micro fungus show that 35 to 72 per cent are nonseptate, and that the remainder are uniseptate or very rarely biseptate. Both light and dark spores are practically all septate at maturity, rarely forming even three septa (see Plate XVII, A–C).

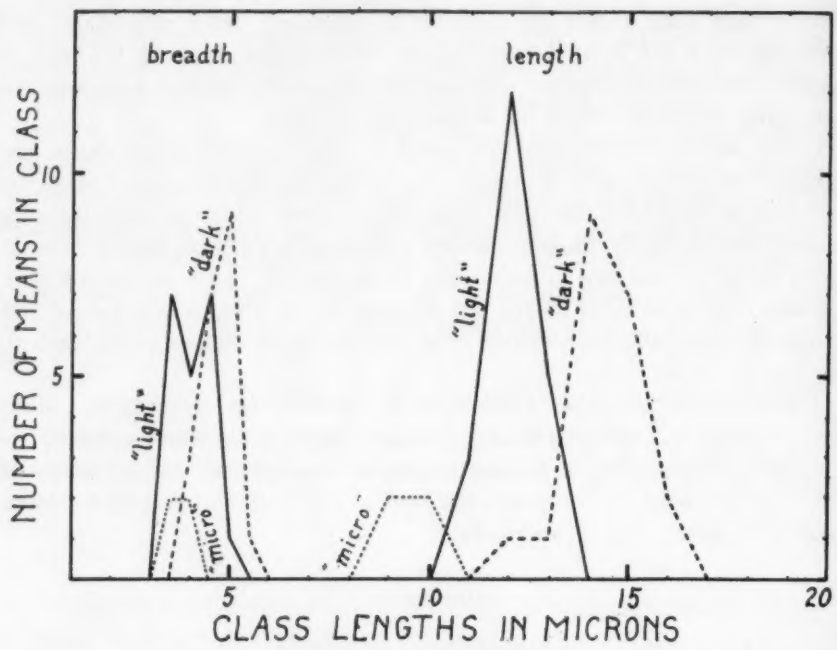

FIG. 1. Distribution of mean breadths and mean lengths of 20 samples of 25 spores each of "light" and of "dark" species, and of 4 samples of 25 spores each of the "micro" *Ascochyta*.

Comparison in Culture[3]

"Light"

Growth is moderate on slants and plates, producing a compact, fluffy, white or very lightly tinted colony, coloring the agar a rich amber or brown. Pycnidia are not produced immediately except in contact with the walls of the culture vessel, but in tubes after 10 days or more, as the agar shrinks away from the glass, a thin mycelial growth covers the new surface and pycnidia usually form in abundance. The pycnidia are brown and they exude spore masses of a clear pink color. With age the mycelium may become matted, and generally assumes the buff color mentioned by several workers. In petri dishes the colonies seldom exceed 7 centimeters in diameter.

Pycnospores are almost cylindrical, slightly constricted, and with rounded ends. They are slender for their length: 11–14 × 3–4 μ; length-breadth ratio 3.7–3.5 (Plate XVII, D).

[3] These descriptions are based on single pycnospore-cultures grown on potato-dextrose agar except when otherwise specified: 200 g. potato, 20 g. dextrose, 17.5–20 g. agar, in 1000 cc. water.

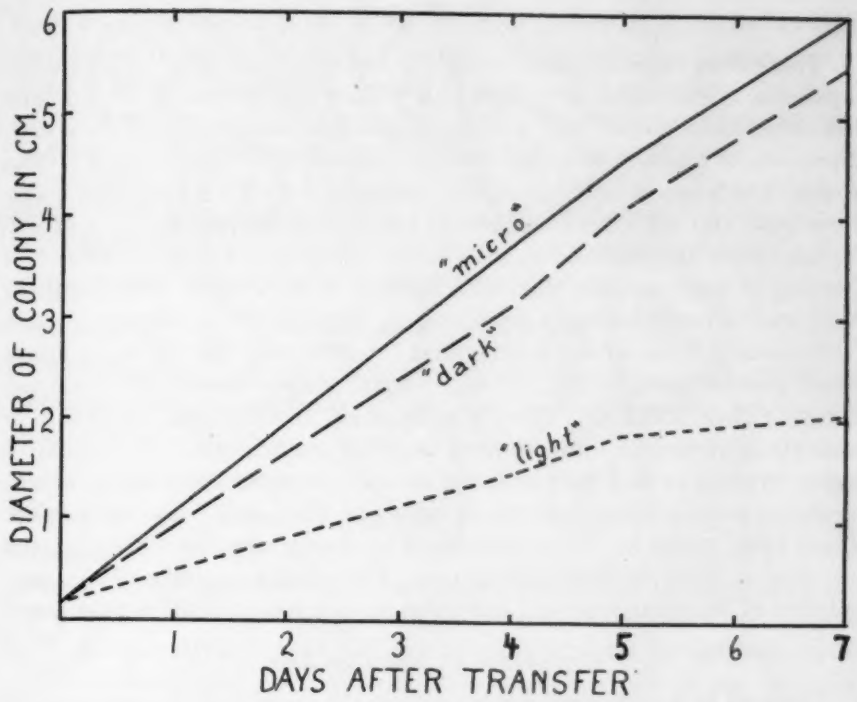

FIG. 2. Average rate of colony growth of the three types of *Ascochyta* on potato dextrose agar in petri dishes at 24° C. Measurements graphed are averages of measurements of 32 isolations of "dark," 16 of "micro," and 4 of "light" fungi.

"Dark"

Growth is more rapid on slants and plates, forming a flat colony at first whitish or light-grey, soon becoming dark olivaceous to black at the center. Pycnidia are produced abundantly after four days at 23° C., arising either from shining, black, radiating strands or scattered in the light mycelium. Pink spore masses are exuded copiously. After this sporulation, a more fluffy olivaceous or sometimes lighter growth spreads over the colony. A few pycnidia are often produced in this fluffy growth, generally surrounded by a mass of dark mycelium, giving old slant cultures a mottled appearance. On plates the dark cultures increase in diameter more than twice as fast as the light, as shown in figure 2. Pigmentation of the potato dextrose agar is almost wholly lacking; or, when developed, blackish but never rich brown.

Pycnospores are relatively plump and distinctly constricted, containing conspicuous oil globules. They measure about 10–13 × 4–5 μ; length-breadth ratio 2.5–2.6 (Plate XVII, E).

"Micro"

This differs from the dark in slightly faster growth (Fig. 2), less aerial mycelium, darker color with more glossy black and olivaceous growth, and less tinted spore masses, but, without microscopic examination of the spores, separation of micro from dark cultures is impossible (Plate XVII, F). The spores, which are usually nonseptate, measure 5–9 × 2.5–4 μ on potato dextrose agar, and 3–6 × 3–4 μ on oatmeal agar. Considerable numbers of uniseptate spores are produced on some media (steamed pea seed). Amount of fruiting is more variable than with light or dark cultures, some isolations from known micro leafspots producing no pycnospores in culture.

Several cultures of the micro fungus, particularly the blacker cultures which produce few pycnidia, develop chlamydospores in great profusion and variety (Plate XVII, G). They may be small, citronshaped, continuous or uniseptate, olivaceous spores formed from the segmentation of irregular or zigzag hyphae, or they may be large, fusiform, oval, or spherical structures of one to three cells, dark-brown in color and filled with large oil droplets. These large spores are borne sometimes in chains, sometimes apically, and are very striking in their appearance. The smaller spores occur in most cultures of the micro type and have been seen in some typical dark cultures.

Crystalline Deposits in Agar

Deposits of two distinct types, apparently crystalline in nature, occur frequently in potato dextrose agar beneath colonies of all three classes of *Ascochyta*. In the substrata of dark and micro cultures there appear compound fan-shaped structures composed of minute, slender, white needles, and resembling strikingly the frost figures formed on window-panes on winter nights. In cultures of the light group, minute golden-yellow specks are formed as far as 8 millimeters below the surface of the agar. Microscopically they appear as spherical asters of fine radiating filaments, some flexuous but others rigid and bent repeatedly at right angles. The dense yellow centers of these asters are generally not in direct contact with the immersed hyphae. Such deposits, though their nature has not been determined, constitute evidence of a close physiological relationship between dark and micro and a distinct separation of these two from the light *Ascochyta*.

Variability and Saltation

In dealing with large numbers of isolations of the three types of *Ascochyta* during a period of 12 months for light and dark and 28 months for micro cultures, variability has been noted especially in both dark and micro cultures, but none of the variants have been seen to approach closely the more nearly constant light type of culture. The dark and micro forms do,

however, show similar types of variation in degree of fluffiness, in color, freedom of fruiting, and rate of growth. Both show the phenomenon of saltation as noted in different fungi by Leonian (12) and others. A single-spore colony may split into sectors of very different appearance and habit of growth, and preliminary work indicates that some of these segregates remain constant through a number of mycelial transfers. These saltations have not yet been seen to involve pycnospore characters, but whether the micro fungus is a well fixed form or merely a frequently recurring saltant or mutant from the dark awaits determination.

INOCULATION EXPERIMENTS

To determine the relative pathogenicity of these three groups of cultures, several inoculation experiments have been conducted on both aerial and subterranean plant parts.

Aerial Parts

In a foliage inoculation experiment begun October 29, 1925, potted peas of Alaska and Horsford's Market Garden varieties were sprayed with pycnospore suspensions from one single-spore culture each of light, dark, and micro types. On December 4, the light fungus had produced typical light spotting; the dark fungus had produced abundant dark-reddish-brown lesions on leaves and stems, some of them zonate and with a few pycnidia; and the micro fungus had formed a few spots similar in appearance to those produced by the dark fungus but without pycnidia. Symptoms were the same on both varieties of peas. Micro spots, when placed in a moist chamber, formed pycnidia with typical small spores. Reisolations yielded, in all cases, cultures indistinguishable from those used for the inoculation.

A much more extensive series was run in August, 1926, using 38 single-spore cultures of the three types obtained from leafspots, footrot, and pea seed. Potted Yellow Admiral peas in the fourth node stage were sprayed with spore suspensions prepared from cultures on potato dextrose agar. One pot of peas was inoculated with each culture, and six pots were retained as uninoculated controls. All were then placed in a spray chamber, where they were held at a temperature of 22–23.5° C. for 64 hours, and then removed to the greenhouse for one day, and returned to the spray chamber at night. They were then removed permanently to the open greenhouse.

The first infection was noted on plants inoculated with dark and micro fungi at the end of 65 hours, when minute, dark-brown flecks were seen on leaves and stems. Inoculation with the light cultures showed no infection until the fifth day, when a few collapsed areas, still green, were noted. On the ninth day, 10 of the 17 pots of peas inoculated with light cultures showed typical stem and leaf lesions, many of them with conspicuous

pycnidia. At this time practically every plant in the 14 pots inoculated with dark cultures and the seven pots inoculated with micro cultures was spotted with numerous points of infection. These infections developed farther, finally showing a complete range of flecks, dendritic spots, zonate spots, and irregular blackening of stems, as observed in the field, all distinctly darker than the definite spots of light type. Dark and micro spotting were indistinguishable. Reisolation yielded cultures of the three fungi indistinguishable from those used for inoculation. The uninoculated controls remained free from spotting throughout.

These experiments show a constancy of correlation between fungus type in culture and type of spotting on the host, and a lack of intermediate symptoms between light and dark, but with micro and dark symptoms indistinguishable. The types of spotting were not influenced appreciably by the three different varieties of peas used in these two experiments.

Footrot Inoculations

Earlier work has indicated that both *Ascochyta pisi* Lib., as previously known, and the micro fungus may cause footrot similar to the Fusarium stem and rootrot of peas caused by *F. martii* App. and Wr., var. *pisi* F. R. Jones, but to compare the pathogenicity of the three fungi here considered the following experiments have been conducted.

In the first experiment, Alaska peas were grown in steamed soil in metal cans held in the Wisconsin soil temperature tanks at soil temperatures of 12°, 20° and 28° C. They were inoculated when planted with crushed cultures of the three fungi grown on steamed pea seeds, one culture tube containing 15 peas per pot. Twenty-eight days from planting date (Oct. 24, 1925) the roots were washed and examined. The light fungus gave no infection at the two lower temperatures, but at 28° C. seven plants or 30.4 per cent were affected with a superficial, yellowish softrot, from which only *Pythium* sp. and bacteria were isolated. The dark and micro fungi both produced well developed footrot symptoms, with the micro evidently causing the more severe injury as well as infecting a larger percentage of plants. The temperature factor appeared unimportant.

A second more extensive experiment was undertaken with 43 different cultures of the three fungi, but owing to the fact that the seed used apparently carried some *Ascochyta*, it need not be reported in detail. Of the plants inoculated with light cultures, 7.6 per cent developed typical footrot, which was less than the 9.1 per cent of footrot in the controls. Dark inoculations gave 63 per cent, and micro gave 87 per cent footrot, both sufficiently higher than the controls to establish the aggressiveness of these two as parasites. The light fungus is, then, clearly less pathogenic if not impotent as a cause of footrot, while both dark and micro fungi may be aggressive in

their parasitism at the base of the epicotyl and top of the taproot of the pea plant.

TAXONOMY

From the foregoing it is apparent that three fungi belonging to the genus *Ascochyta* Lib. are of common occurrence as parasites upon *Pisum sativum*. Two of these, designated dark and micro, have many characteristics in common, while the third, the light, is so widely different from them both on the host and in pure culture that it merits specific rank by itself. In the past, at least two of these, dark and light, have been listed in both literature and exsiccatae under the name of *Ascochyta pisi*.

This name was given in 1830 to a collection on pods of *Pisum sativum* from France, distributed by Madame Libert (13) with a brief description on the packets. This description, and also symptoms and spore measurements (11–15 × 3–4 μ; mean, 12.7 × 3.5 μ) of a packet of Libert's collection in the Farlow Herbarium at Harvard University identify this with our light fungus.

Descriptions of presumably the same fungus were published shortly afterward by Link, Berkeley, and others. Link's (15) description is brief and follows Libert's closely. Berkeley (1) described spots on both pods and leaves which are certainly of the light type under the name of *Sphaeria* (*Depazea*) *concava* Berk., but in 1860 (2) he listed this as a synonym of *Ascochyta pisi*. Meantime, in 1859, he had published *Sphaeria* (*Depazea*) *pisicola* Berk. (3) as a new species with a brief description including the spore length of 0.0004–0.0005 inch (10–13 μ), which identifies this also with our light fungus. What distinction Berkeley saw between his two species is not revealed in his descriptions. *Ascochyta pisicola* (Berk.) Sacc. is thus a synonym of *A. pisi* Lib. as suggested by Stone (19), and our light fungus is this species.

Descriptions of *Ascochyta pisi* usually given, as those of Saccardo (17), and Davis (4), are based upon the light type of spotting, this apparently being regarded as the typical development of the species, but the spore measurements most commonly given (14–16 × 4–6 μ) are larger than the largest sample we have obtained from a light spot and agree more closely with our measurements of the dark fungus.

Both light and dark fungi were present in Europe as early as 1895, for the fungus described by Krüger (11), and Hiltner (8) as the cause of serious footrot and seedling blight was clearly the dark species, and both are present in a packet of Krieger's Fungi Saxonici 1548 collected in 1895. In America, Van Hook's (20) illustrations, descriptions, and spore measurements indicate that he met with both types, and Stone (19, p. 565) described both without differentiating.

Krüger (11) first suspected what was later considered established by Stone (19) and Vaughan (21), that *Ascochyta pisi* as then understood is a pycnidial stage of *Mycosphaerella pinodes*. This work appears to have been done, however, with our dark species and not the true *A. pisi*.

Krüger was dealing with a virulent footrot parasite which turned the vines black. Stone's paper indicates that it was the dark fungus with which he was chiefly concerned, and Vaughan has personally identified our dark fungus as his *Mycosphaerella pinodes*. In addition to these lines of evidence we have found perithecia with mature ascospores of this *Mycosphaerella* in some dark cultures on Melilotus stems, and single ascosporous cultures from these have produced only the dark culture. The dark fungus and probably not *A. pisi* is thus the pycnidial stage of *M. pinodes*.[4]

With our dark fungus thus placed as the imperfect stage of *Mycosphaerella pinodes*, it requires description[5] to distinguish it from *Ascochyta pisi*. This latter fungus, now left unattached to a perfect stage, regains its former designation, but the description requires slight emendation. The micro fungus will be described tentatively for convenience without attempting to give it final taxonomic treatment.

Ascochyta pisi Lib.

Spots definite, circular on leaves and pods, elongate on stems, yellowish-brown with dark-brown margin often prominent, sometimes whitish in center, spots not defined on mature plant parts; pycnidia gregarious at center or in circular zones, somewhat prominent, depressed-globose, light to dark-brown, ostiolate, 75–225 μ; spores hyaline, oblong, ends rounded, straight or somewhat curved, 1–(1–3) septate, somewhat constricted, 10–14 (8–19) × 3–5 (2.5–6) μ, mean length less than 13.5 μ.

On leaves, stems, pods and seeds of *Pisum sativum* L.

Mycosphaerella pinodes (Berk. and Blox.) Stone.

Pycnidial Stage.—Spots irregular to circular-zonate, not definitely margined, dark-brown, sometimes lighter when zonate, spots not defined on mature plant parts; pycnidia scattered, rarely at center, somewhat prominent, irregularly depressed globose, light to dark-brown, indistinct in spot, ostiolate, 65–175 μ; spores hyaline, oblong, ends rounded, straight or somewhat curved, 1–(1–3) septate, sharply constricted, 12–17(10–21) × 3.5–5.5(3–7)μ, mean length more than 13 μ.

[4] The following abstract presenting evidence in essential agreement with ours that *A. pisi* and *M. pinodes* are two separate organisms appeared after this paper had been submitted for publication.

Jones, L. K. The relation of *Mycosphaerella pinodes* to *Ascochyta* blight of peas (Abst.). Phytopath. 17: 44. 1927.

[5] The description given by Stone (19, p. 581) is too general to serve diagnostically.

On leaves, stems, pods, and seeds of *Pisum sativum* L. Also causes firm, dark-brown rot of epicotyl and upper taproot.

Mycosphaerella pinodes (Berk. and Blox.) Stone, micro form?

Similar to pycnidial stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone; spores continuous to 1-septate, continuous spores oval to oblong, 5–11 × 2.5–4.5 μ, uniseptate spores oblong, rounded at ends, more or less constricted at the septum, 8–12 × 3–4.5 μ.

On leaves, stems, pods, and seeds of *Pisum sativum* L. in Wisconsin. Also causes firm, dark-brown rot of epicotyl and upper taproot.

This revision of the old conception of the species *Ascochyta pisi* throws into uncertainty work done hitherto on the host range of this species. Scalia's (18) attempt to mass the legume-infesting species of *Ascochyta* under this name, upon morphological bases, seems now especially in need of review. Very little can yet be said as to the identity of the species of *Ascochyta* reported on a considerable range of species of *Vicia*, *Lathyrus*, etc. In Wisconsin, an *Ascochyta* spot, referred to *A. pisi*, is of common occurrence on *Vicia angustifolia*, and the type of spotting is rather similar to the authentic *A. pisi* on peas. Further, the fungus isolated from such spots from three separate localities agrees in bearing very close resemblance in general appearance to *A. pisi* and in being totally unlike our cultures of *Mycosphaerella pinodes*. No detailed study of the fungus on vetch has been made. Further study should attempt to find the perfect stages of *A. pisi* and of the micro fungus, if any, and to determine the range of variability and parasitism of these three fungi.

SEED INFECTION

It is known from the work of Halstead (7), Krüger (11), Hiltner (8), Van Hook (20), and others that *Ascochyta pisi*, as formerly understood, is carried freely on pea seed. In the present study numerous samples of seed have been tested[6] to determine which of the three fungi here distinguished are thus carried.

Transfers to slants, from 94 samples of Wisconsin and Eastern grown seed thus tested, included all three of these *Ascochyta* forms and some other fungi. Twenty-eight samples yielded *A. pisi*; 23, *M. pinodes*; 5, the micro fungus (9 samples contained two or three of these fungi); and 8 other infested samples were not classified. Not only was *A. pisi* most frequently

[6] A sample of peas (50 or 100) was immersed 30 seconds in 70 per cent alcohol, transferred to 0.1 per cent $HgCl_2$ for 1 minute, and washed for 2 hours in three changes of sterile water. The peas were then drained and planted, six to eight in a petri dish, pressed down into a deep layer of 1 per cent agar, acidified with lactic acid. Potato dextrose was generally used, but Dox's medium was used with success in some instances.

found in the samples, but these samples also contained a somewhat higher average percentage of infected seeds than the samples in which *M. pinodes* or the micro fungus was found. This is significant in view of its negligible importance as a cause of footrot. The total of 54 infested samples showed an average of 8.4 per cent of seeds affected with these three fungi; the highest percentage in one sample was 33, but several tested 20 per cent or more. These fungi occurred in samples from widely separated localities in Wisconsin and, collectively, in the following varieties of peas: Alaska, Winner, Yellow Admiral, Green Admiral, Advancer, Perfection, Horsford, Ashford, Horal, Badger, Rice's 13, Prince Edward, and some unnamed selections.

PLOT TESTS

From the above seed tests, 24 samples of infested seed, together with 7 samples of *Ascochyta*-free Wisconsin and eastern-grown seed and 19 samples of clean Idaho-grown seed representing several varieties, were selected for a plot trial to learn something of the significance of seed-borne species of *Ascochyta*. Two hundred seeds of each of these samples were planted in duplicate in rows 3 feet apart, the seeds about 1 inch apart in the row. Planting was begun May 14, somewhat later than the middle of the planting season for canning crop peas. Rains forced a delay in planting, and the remaining peas were planted May 20–22 while the surface soil was wet and sticky. When the early varieties were beginning to bud, from June 18 to 22, one of the duplicate rows of each sample was dug, counted, and examined for footrot symptoms. The other row was left standing to maturity for a determination of Ascochyta spotting of the vines.

The plants, as dug, were notably clean, comparing favorably in appearance with what may generally be noted in late sown commercial fields. The rows planted from badly infested samples appeared, in general, as clean as the controls, all of them bearing more or less frequent lesions caused by *Rhizoctonia*. To check upon minor differences, calculations were made of percentage of stand, percentage of plants infected, percentage of plants cut off below ground and sprouted again, and infection-rating, as summarized in table 1, but no significant correlation with infested seed was found except in the case of percentage of stand. Infested samples as a group gave poorer stands by a percentage figure which agreed closely with the average percentage of infected seeds in the samples, but individual samples fluctuated widely from this average correlation. No significant differences between *A. pisi*, *M. pinodes*, or mixtures of these with the micro fungus could be detected. Since germination tests of the seed used in this plot work were not made, this reduction of stand noted in infested samples cannot be analyzed precisely.

The other interesting result of this trial was that the early planting gave better stands than the late; the infested samples in the early planting did as well as the controls planted one week later under the different environmental conditions then prevailing.

Aside from these differences in stand, the infested samples were practically indistinguishable from the controls, showing, if anything, slightly lower percentages of footrot and lower infection ratings. Obviously there was no significant influence of the seed-borne fungi in this one season trial other than possibly that of reduced stand.

The duplicate rows in this trial plot came to a normal maturity and developed only tardily a slight trace of leaf spotting.

TABLE 1.—*Summary of data from plot test of seed infested with Ascochyta species compared with Ascochyta-free seed*

Planting dates	Seed samples		No. of rows of 200 seeds	Seeds infected per cent	Stand per cent	Plants infected per cent	Footrot infection rating[a]
May 14, 1926	Controls, Idaho grown		9	....	88.5	36	7.8
	Infested	*A. pisi*	4	13	78.8	19	3.7
		M. pinodes	3	3.3	86.6	25	3.6
		Mixed	2	7.5	71.7	24	5.4
		Undetermined	2	6.5	80.7	45	16
	Total infested samples		11	8.2	80	27	6.2
May 20–22, 1926	Controls	Idaho grown	10	....	79.4	29.3	7.7
		Wisc. grown	7	....	81.8	30	6.8
	Total controls		17	....	80.4	30	7.3
	Infested	*A. pisi*	2	11	47.2	29	7.8
		M. pinodes	2	8	79.7	38	6.1
		Mixed	6	12.3	70.3	24	3.7
		Undetermined	3	6	72.3	38	8.9
	Total infested samples		13	10	68.6	30	5.9

[a] Method of calculation modified from McKinney (16).

FIELD OBSERVATIONS

A continuation of the Wisconsin pea disease survey in 1925 found Ascochyta spotting more prevalent than in 1924 (9), occurring in 9.4 per cent of the 700 fields surveyed. In several instances it clearly was seedborne. For example, one lot of Pedigreed Extra Early peas imported from a humid region was planted in two widely separated counties in Wisconsin. In both places every field planted from this seed developed the light Ascochyta leafspot, while no such spotting could be detected in fields planted from other

seed stocks in the same localities. Elsewhere in the State, similar spotting was seen in a number of lots of both canning and field varieties from Wisconsin seed, but none of these fields bearing the light *Ascochyta* (*A. pisi*) suffered any marked injury. The few instances of moderately severe injury from *Ascochyta* observed in the survey were in fields where peas were planted immediately following peas without rotation and where the dark Ascochyta leafspot (*Mycosphaerella pinodes*) was present. However, during the past three years in Wisconsin serious injury from *Ascochyta* species has been all but absent, and both bacterial blight (*Bacterium pisi* Sackett) and anthracnose (*Colletotrichum pisi* Pat.) have been more important foliage diseases.

Peas grown for canning in several localities in Wisconsin from seed tested and found to contain one or both species of *Ascochyta* were observed through the season of 1926. A lot of Perfection peas which tested 24 per cent *Ascochyta*, both *A. pisi* and *M. pinodes*, developed small and varied amounts of dark Ascochyta spotting on leaves and stems in five fields observed, as well as footrot (6, 29, 13 and 8 per cent) in four of them. A few plants were markedly weakened, but yields were excellent and quality of peas very good. In the same locality two fields planted from clean Idaho grown Perfections showed 17 and 27 per cent footrot (micro fungus isolated); both fields had grown several crops of peas prior to 1923.

A lot of Advancer seed, tested at another experiment station and reported to the canner as "96 per cent germination, 12 per cent *Ascochyta*," produced plants 10 per cent of which showed slight traces of both light and dark Ascochyta spotting and a mere trace of footrot.

Several fields planted from tested lots of Yellow Admiral seed with as high as 18 per cent *Ascochyta* were followed in two localities. All showed more or less light leafspot but no footrot. In one field the plants weakened by Aphanomyces rootrot were badly mutilated by deep stem and leaf lesions, many plants being entirely cut off above the ground; in other fields the spotting was of little consequence.

Footrot was noted in several localities where leaf spotting was entirely absent in fields which had grown several crops of peas before. One such field had practically 100 per cent of footrot, although peas had not been grown for two years. Isolations in such cases yielded usually either the micro fungus or *Fusarium martii* App. and Wr. var. *pisi* F. R. Jones.

These field observations indicate that species of *Ascochyta* were of no great importance as pea parasites in Wisconsin during 1925 and 1926, and at the same time throw some light upon factors which determine the field occurrence of these fungi. Light spotting, produced by *Ascochyta pisi*, was very clearly and closely correlated with the planting of infested seed. Dark spotting was correlated with the occurrence of *Mycosphaerella pinodes* on

the seed planted, and also with the former growth of peas in the field. This fungus shows more marked tendency to accumulate in the field with repeated cropping; probably the perithecial stage functions importantly in overwintering. It is interesting to note that the serious outbreaks of *Ascochyta* on peas recorded in the literature appear to have been concerned with this rather than with the authentic *A. pisi*. Footrot was found to be correlated with the occurrence of either the micro fungus or *M. pinodes* on the seed, but also, and perhaps more importantly, with the previous growth of peas in the field, even beyond a two-year interval between crops. In this behavior the micro fungus agrees with the other pea rootrot parasites. In culture this fungus behaves as a somewhat better saprophyte than either of the species, and its stronger tendency to produce large chlamydospores with a reduced pycnospore production suggests a better adaptation to subterranean existence.

Disease does not necessarily result from the planting of infested seed. The plot trials reported in this paper, as well as the practical field experience of Wisconsin pea growers, demonstrate that seed carrying any of these three fungi may be planted, under some conditions, without the development of an appreciable amount of either leafspot or footrot. On the other hand, it is certainly preferable to plant seed which is known to be free from *Ascochyta* when such seed is available. Seed bearing *Ascochyta pisi* is less to be feared than seed which carries either *Mycosphaerella pinodes* or the micro fungus.

It is clear, from our own observations and from records in the literature, that weather conditions prevailing during germination and the growth of the crop determine very largely the extent and severity of development of disease from infested seed. A detailed study of the influence of environmental factors upon various phases of the parasitism of these fungi should go far in explaining the irregular behavior of the species of *Ascochyta* parasitic on the pea.

SUMMARY

1. Two species and a provisionally designated form of *Ascochyta* are reported as parasitic on the pea (*Pisum sativum* L.). Two of them have been regarded formerly as *Ascochyta pisi* Lib., the imperfect stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone.

2. The fungus here regarded as *A. pisi* differs from the imperfect stage of *M. pinodes*: (a) in size and shape of pycnospores; (b) in character of the spot produced on the host; (c) in length of incubation period on aerial parts; (d) in the ability of *M. pinodes* but not of *A. pisi* to cause footrot; (e) in rate and character of growth and sporulation in pure culture on artificial media; and (f) in the failure of ascospores of *M. pinodes* to produce *A. pisi* in culture.

3. A third fungus, related to the imperfect stage of *M. pinodes* and here referred to as the "micro" fungus, is characterized by small and commonly nonseptate pycnospores. It is a frequent cause of footrot, and may also produce leafspots which are indistinguishable from those caused by *M. pinodes.*

4. Emended descriptions are given of *A. pisi* and the pycnidial stage of *M. pinodes.* The "micro" fungus is described under the tentative name, *Mycosphaerella pinodes* (Berk. and Blox.) Stone, micro form?

5. *M. pinodes* rather than *A. pisi* seems to have been the cause of the more severe outbreaks of the Ascochyta disease on peas reported in literature. Inoculation studies and field observations recorded here indicate that this is a more serious pathogen than *A. pisi.*

6. Seed tests have shown that all three of these fungi are carried on pea seed. *A. pisi* was most frequently present in the samples tested during 1926.

7. The planting of infested seed may serve to establish any of these fungi in the resulting crop of peas, but the extent and severity of the resultant injury may vary widely with environmental conditions.

8. In one season's plot trials, seed infested with these fungi gave, on the average, poorer stands than clean seed, but resulted in no considerable footrot or leafspot.

9. Field observations indicate that *M. pinodes* survives Wisconsin winters better than *A. pisi,* and is more likely to increase with intensive pea culture. The "micro" fungus may persist in the soil for more than two years between crops of peas. The occurrence of *A. pisi* is more closely associated with the use of infested seed.

10. Extended field observations indicate that the diseases caused by species of *Ascochyta* were of minor importance in pea culture in Wisconsin during 1924, 1925 and 1926.

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EXPLANATION OF PLATES

PLATE XVI

Photographs of naturally occurring leafspots caused by "light" (*Ascochyta pisi*) and "dark" (*Mycosphaerella pinodes*) species of *Ascochyta* on leaflets of cultivated pea. All photographed ×8. A, B, and C. "Light" leafspots. D, E, F, and G. Types of "dark" leafspots.

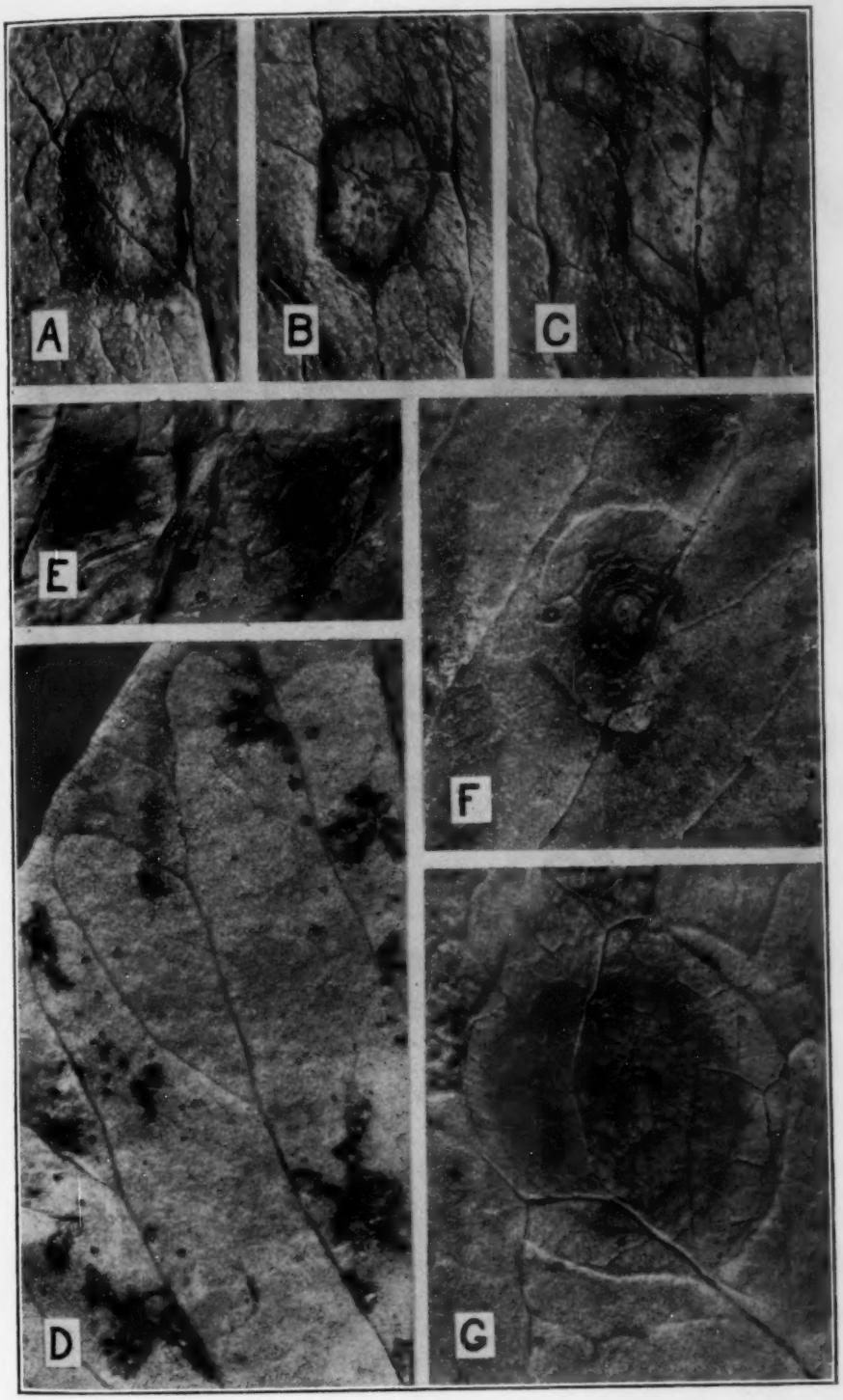
PLATE XVII

Camera lucida drawings of spores of the pea-infesting species of *Ascochyta*, ×1100.

A, B, and C. Pycnospores of "light" (*A. pisi*), "dark" (*M. pinodes*), and "micro" fungi respectively, from naturally occurring spots on pea leaflets.

D, E, and F. Pycnospores of "light," "dark," and "micro" fungi, respectively, from cultures on potato dextrose agar.

G. Types of chlamydospores from culture of "micro" fungus on potato dextrose agar.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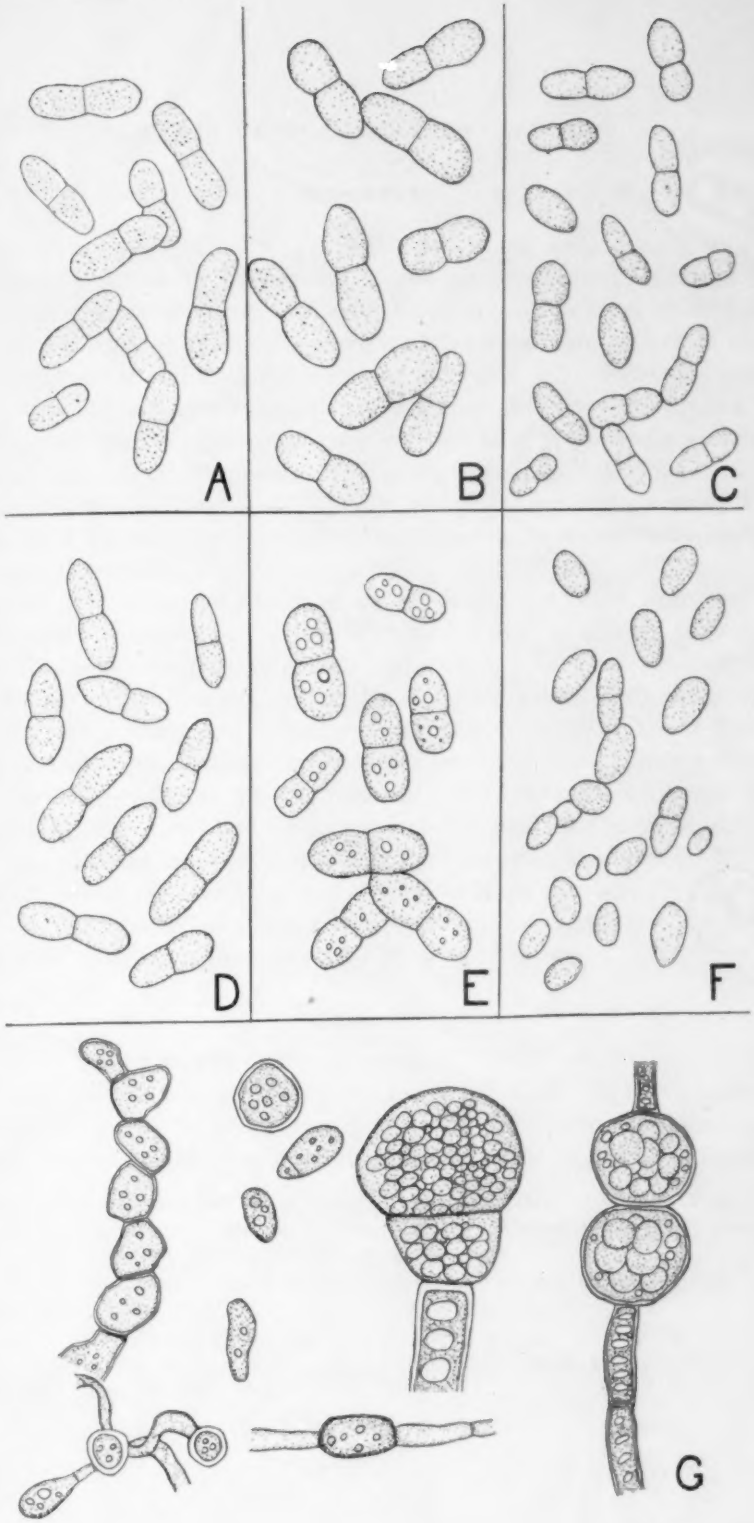
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LEAFHOPPER INJURY TO CLOVER[1]

E. A. Hollowell, John Monteith, Jr., and W. P. Flint

Throughout most of the clover belt a yellowing, reddening, browning, and generally unthrifty appearance of red clover plants (*Trifolium pratense*) is commonly observed. This condition usually becomes evident soon after the first cutting of clover is harvested, becomes more noticeable as the summer advances, and disappears during the early fall. Several attempts have been made to isolate a causal organism from the browned portions, but no fungous or bacterial parasite has been found to be consistently associated with these particular symptoms. The browning of the leaves has been frequently ascribed to "sun scald," while the yellowing and dwarfing have usually been regarded as a physiological response to unfavorable climatic conditions of summer.

The tip and marginal browning of the leaves has often suggested the similar injury found on potato plants which has been attributed to leafhoppers.[2] The accompanying yellowing and dwarfing suggests the symptoms of "yellows" or "yellow top" of alfalfa which in certain parts of the country is popularly attributed to leafhoppers. Piper,[3] in 1914, noted that "a species of leafhopper seems to be constantly associated with alfalfa yellows." However, this correlation was not confirmed by experimental methods until the recent tests by Jones and Granovsky[4] (work conducted independently at Wisconsin simultaneously with the work here reported on clover). Several species of leafhoppers are frequently found in large numbers feeding upon clover, but no experimental work has been reported heretofore which correlates these symptoms on clover with leafhopper injury.

SYMPTOMS

The leaf injury on red clover, as commonly found, consists of a distinct tip or marginal browning, where the tissue is killed. The "tipburn" is ordinarily V-shaped, with the apex of the V on the midrib, and bounded by the veins extending from this point to the margin. Such dead segments

[1] Investigations conducted cooperatively between the Offices of Forage Crops, Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Illinois Natural History Survey.

[2] Ball, E. D. The potato leafhopper and the hopperburn it causes. Wis. Dept. Agr. Bul. 23: 76–102. 1919.

[3] Piper, C. V. Forage plants and their cultures. 618 pp. New York. 1914.

[4] Jones, Fred R., and A. A. Granovsky. Yellowing of alfalfa caused by leafhoppers (Abs.). Phytopath. 17: 39. 1927.

are generally distinctly curled. A similar, though smaller, browned area may occur anywhere along the leaf margin. Frequently the entire leaf may wilt, dry out and become brown. At times, instead of this browning, the affected leaves may become yellowed or bronzed, as in alfalfa "yellow-top," this symptom being particularly common in some of the European strains of red clover. There is often a distinct distortion of the stems and petioles. When growing parts are affected, development is decidedly checked and in severe cases the plants are killed.

In the early summer of 1926 plants of red clover which had just reached the blossoming stage in pot cultures at the University of Illinois were found to be generally affected with this marginal and tip burning of the leaves, together with the yellowing and dwarfing. It was observed that these plants were heavily infested with leafhoppers, mainly the potato leafhopper (*Empoasca fabae*, Harris). The abundance of these insects and the close similarity between symptoms manifested on these clover plants and those described as hopperburn on potatoes further served to support the supposition that the tip and marginal browning of these two hosts might be attributed to the same cause. In field observations near Urbana, Illinois, it was noted that, where this type of injury was most noticeable, leafhoppers were usually most plentiful. As a result of these observations, experiments were conducted in insect-proof cages to determine whether this condition of clover was actually due to leafhopper injury.

FIRST TEST—URBANA, ILLINOIS

Four red clover plants of the Tennessee anthracnose-resistant strain, F. C. I.[5] No. 2469, and two plants from English seed, S. P. I.[6] No. 61333, were selected as nearly alike as possible in regard to vigor and growth. These plants, which were approximately 8 months old, were sprayed with nicotine sulphate and examined to make sure they were free from insects before placing them in wire insect-proof cages (Fig. 1) on July 20. Two of the Tennessee and one of the English plants were placed in each of two cages. The following day 20 leafhoppers (*Empoasca fabae*) in different stages of development were placed in Cage 2, and on July 27 a few additional hoppers of the same species were inserted to replace some that had died. The cages were examined frequently to observe any injury to the plants and to see that no leafhoppers were present in the insect-free cage.

On August 2, differences were first noted between the plants of the two cages. In Cage 2 several leaves had died, many others were browning and the plants in general lacked vigor and appeared stunted. It was noted that

[5] Accession number of the Office of Forage Crops, Bureau of Plant Industry, United States Department of Agriculture.

[6] Accession number of the Office of Foreign Seed and Plant Introduction.

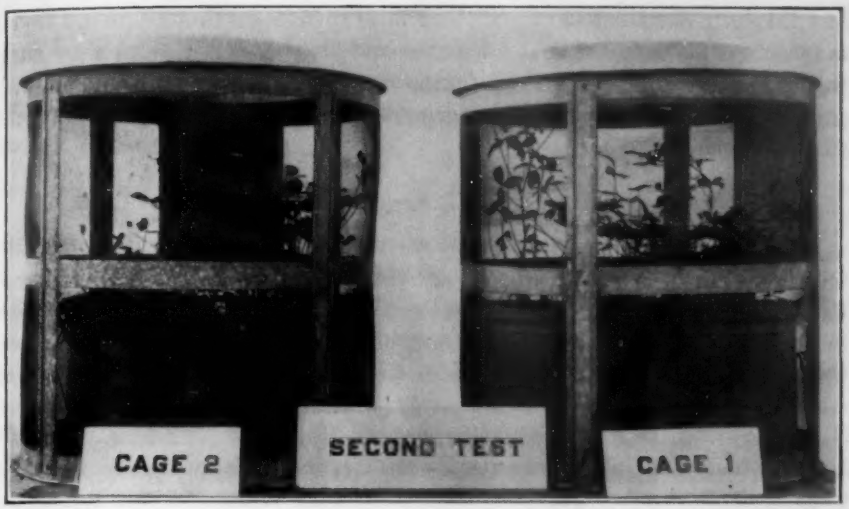

FIG. 1.—Cages used in the experiments at Urbana, Illinois, to test the effect of potato leafhoppers on red clover.

the relatively glabrous English clover was injured to a greater extent than the Tennessee plants. The plants in Cage 1 showed no injury, whereas the extent of damage to those in Cage 2 increased rapidly. On August 19, the plants from both cages were removed and photographed (Fig. 2). Those from Cage 1, which had all remained entirely healthy and had bloomed, were examined for leafhoppers, but none was found. Plants 2 and 3, from the leafhopper-infested cage, were dead. No. 1 had a few green leaves,

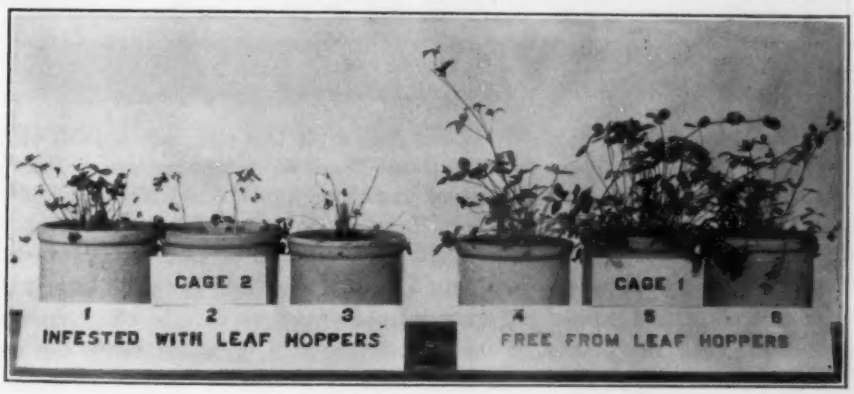

FIG. 2.—First Test. Red clover plants from cages free from, and infested with, leafhoppers. Plants 1, 2, 5, and 6 are from Tennessee anthracnose-resistant seed, F. C. I. No. 2469. Plants 3 and 4 are from English seed, S. P. I. No. 61333.

but did not make further growth, and later died, even though placed under favorable conditions. The dead leaves and stems of each plant were carefully examined for bacteria and fungi, but, except for an apparently saprophytic species of *Alternaria*, no organism was found consistently in the affected tissue.

SECOND TEST—URBANA, ILLINOIS

In order to substantiate the evidence obtained from the first test, the experiment was repeated. On August 19 the three plants from Cage 1, which was free from leafhoppers in the previous test, were placed in Cage 2 to be infested with hoppers in the second test. Three similar plants, from the same seed sources, were placed in Cage 1 to be kept free from insects. About 20 leafhoppers (*Empoasca fabae*) were placed in Cage 2 on August 20, and during the first week of September 10 more were added, for many hoppers had died in the meantime. A mature leafhopper was found in Cage 1 on September 22, and when the plants were removed for photographing on October 2, two mature hoppers and several nymphs were found, showing that the plants therein had not been completely free from hoppers. Plants 2 and 3 from the infested cage were dead, and a few dead stems and leaves were noted on No. 1 (Fig. 3). Plants 4 and 5 showed no injury of

FIG. 3.—Second Test. Red clover plants from cages free from, and infested with, leafhoppers. Plants 1, 2, 5, and 6 are from Tennessee anthracnose-resistant seed, F. C. I. No. 2469. Plants 3 and 4 are from English seed, S. P. I. No. 61333.

any kind, but a few stems and leaves had been killed on plant 6, indicating that the leafhoppers which were found in this cage had been feeding only on one plant or had gained entrance into the cage too late to affect results materially. All the plants in the second test had been cut twice during the season and were less vigorous than those in the first test. Observations made in the second test confirm those in the first, in that the smooth succulent English plants appeared to be injured sooner than those of the hairy American type.

The plants of the potted series, which were not used in these experiments, were sprayed with bordeaux mixture. It was found that, when thus protected, the plants remained comparatively free from leafhoppers, and were much more vigorous and healthy than when heavily infested with the insects.

OBSERVATIONS AT ARLINGTON FARM, VIRGINIA

During the summer of 1926 the same type of injury was observed on the plantings of red clover at Arlington Farm, Virginia, where several species of leafhoppers were abundant. The tip and marginal browning was common on plants grown from seed produced in various foreign and American localities. In general, it appeared that the relatively glabrous foreign clovers were more seriously affected than were the pubescent native strains. Yellowing and dwarfing were much more conspicuous in the plots sown with Italian seed. In these plots the mortality also was greater, but since the Italian strain is more susceptible to certain diseases, and is less vigorous during the summer months than the native strains of red clover, the actual relative losses due to hopper injury have not yet been definitely determined.

In the red clover plots planted with Italian seed there is usually a large number of alfalfa plants. Throughout the summer it was observed that these alfalfa plants were much dwarfed and showed the typical symptoms of "yellows" or "yellow top." Adjacent plantings of alfalfa from seed of different origin were heavily infested with leafhoppers and the plants were stunted and yellow. Several inquiries received by the Department of Agriculture from different eastern localities indicated that alfalfa "yellow top" was more conspicuous than usual during the summer of 1926. This was probably due to the presence of leafhoppers in numbers greater than usual.

The yellowing, bronzing and dwarfing found typically in alfalfa were more pronounced in the Italian red clover than in the native American clovers in which the leaf-browning symptoms predominate. Leafhoppers appeared to be somewhat more numerous on the glabrous Italian red clover and on the alfalfa in these plots than on the plants of the pubescent American strains in adjoining plots. Whether the more striking symptoms manifested in these plants are due to some preference of the leafhoppers for these strains or to greater susceptibility of the plants to this injury was not determined. Plants of Italian and native clover were caged at Arlington, but the differences were not sufficiently striking to warrant any definite conclusions. This failure was probably partially due to the fact that the plants showed some symptoms of injury at the time they were caged and these symptoms persisted in the insect-free cages, and also due to the death

of the majority of the caged leafhoppers. However, there were differences which supported the results of the experiments at Urbana, Illinois.

INJURY ON OTHER CLOVERS

On other species of *Trifolium*, including *T. medium*, *T. hybridum*, *T. repens* and *T. incarnatum*, symptoms similar to those on red clover are commonly in evidence during the summer months and could be associated with large numbers of various species of leafhoppers. The symptoms vary somewhat with the species; in *T. medium*, for example, the tip burn is much more conspicuous, whereas in *T. hybridum* the yellowing or bronzing of the leaves is most noticeable. Other legumes used as forage crops frequently exhibit similar yellowing or tip burning and, since leafhoppers are usually numerous on such crops, it may be assumed that this condition is caused in large measure by these insects.

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THE FLAGELLA OF BACILLUS AMYLOVORUS

MARY K. BRYAN

In a recent publication[1] Dr. H. R. Rosen questions the peritrichiate flagellation of the fire-blight organism, *Bacillus amylovorus,* and figures polar flagella as demonstrated by a stain of his own devising. He claims that the bacteria with which he worked were isolated by him from typical fire-blight lesions, agreed with *B. amylovorus* in cultures, and produced infections on young pear fruits.

Again in a later paper[2] Rosen says "Likewise it has been determined that the germ causing the disease known as fire-blight of apples and pears has usually but a single polar flagellum and in no case has it been found to have appendages arranged around the body of the organism."

In the summer of 1926 the writer obtained *Bacillus amylovorus* from blighted twigs sent by pathologists from as widely separated places as New York, Illinois, Georgia, Maryland and the District of Columbia. Three of these were on pear, two on apple and one on *Crataegus.*

The flagella of organisms from these six isoiations have now been stained by the method of Casares-Gil. A colony which proved very virulent when inoculated into pear twigs was used in each case.

The results are shown in plate XVIII. As with other capsulate organisms it has been difficult to get good clean slides. Peritrichiate rods were always numerous on every slide stained from all sources. Some rods, however, show only one flagellum. These are easy to find on preparations of any multiflagellate pathogen just as many detached flagella and many rods with no flagella are commonly present.

It seems probable, therefore, that Rosen either did not have the right organism or that his bacteria lost most of their flagella in the process of staining.

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[1] Rosen, H. R. The number and arrangement of flagella of the fire blight pathogen, *Bacillus amylovorus.* Mycologia **18**: 23–26. 1926.

[2] Rosen, H. R. Plant Diseases: Morphology and life cycles of bacteria. 38th Ann. Report, Arkansas Agr. Exp. Sta. Bul. 215: 58. 1926.

EXPLANATION OF PLATE XVIII

Bacillus amylovorus from six sources, stained by Casares-Gil's flagella stain. Note the capsules. Photomicrographs by James F. Brewer.

A. Pear twig from New York.

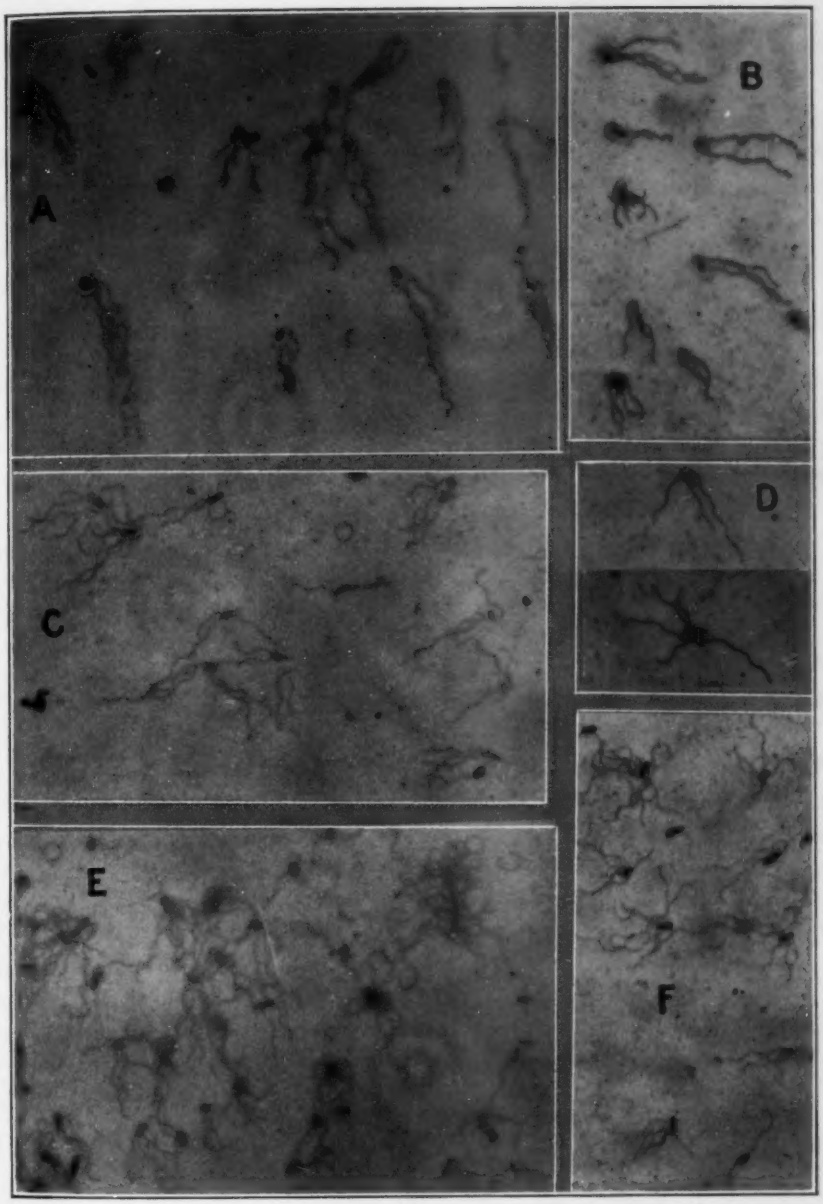
B. Apple twig from New York.

C. *Crataegus* from the District of Columbia.

D. Pear twig from Georgia.

E. Pear twig from Illinois.

F. Apple twig from Maryland.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THE CURLY TOP OF SUGAR BEET IN THE ARGENTINE

G. L. Fawcett

When the curly top of sugar beet was first noticed in the Argentine a few years ago, it was taken for granted that it was the same as the North American curly top, and it was believed that *Eutettix tenella*, which transmits the disease in that country, would occur here. However, no insects agreeing closely with the description by Ball[1] were to be found. The two species of leaf-hoppers differing least from *E. tenella* were sent to the taxonomist for this group at the California Academy of Science and to the United States National Museum. In a reply received from the latter institution, it was stated that one of the insects was a dark form of *Aceratogallia sanguinolenta* Prov., and the other, *Eutettix* sp. Meanwhile the so-called *A. sanguinolenta*, gathered on diseased beets, had been shown to transmit the infection of curly top to healthy beets grown under cages. The beets under cages into which the *Eutettix* sp. and other insects were introduced remained healthy, as did the checks. A description of this work was published in 1925.[2] Recently a letter was received from Dr. Herbert Osborn, to whom specimens were sent not long ago, in which it was stated that my *A. sanguinolenta* is really *Agallia sticticollis* Stål., a distinctively South American insect; it would now be placed in the *Aceratogallia* of the later classification.

No doubt it is because of the interest taken in curly top in North America, where it causes much loss to the sugar industry, that my paper received unexpected attention. The fact that the transmission of the disease was attributed to *A. sanguinolenta*, an insect proved to have nothing to do with the transmission of curly top in the United States, must have seemed rather startling. A letter was received from Dr. Eubanks Carsner, in which he suggested the desirability of a more authoritative determination of the insect, which we proceeded to obtain. He also expressed his belief that *E. tenella* was the real cause of our curly top, kindly supplying photographs and descriptions which would have made it difficult not to recognize that insect had it occurred here. Of course the accidental introduction of *E. tenella* into the cages along with other insects would have furnished sufficient explanation of the occurrence of the disease in the cages. But this

[1] Ball, E. D. The leaf-hopper of the sugar beet. U. S. Dept. Agr., Bur. Entom. Bul. 66. 1909.

[2] Fawcett, G. L. Encrespamiento de las hojas de la remolacha azucarera. Revista Industrial y Agrícola de Tucumán **16**: 39–46. 1925.

criticism does not take into account the relative ease with which the different species of leaf-hoppers can be distinguished from each other, even with the naked eye. My practice has been to collect the insects a few at a time, in a test-tube. In the case of the earlier experiments the insects were then examined with a hand lens before putting them into the cages. This year the insects have been etherized in the test-tube, and then removed and examined separately with a microscope of a magnification of about 30 diameters. I do not know that etherizing has been used before in such work. It facilitates examination of the insects and, if prolonged exposure is avoided, does not injure them.

The experiments carried out this season have confirmed the results obtained previously. In addition to the larger cages of cloth and wire, under which the beets were planted and grown, we have also made use of smaller cages, each covering a single plant in a pot. In all cages into which the *A. sticticollis* has been introduced, curly top has developed; the plants in the check cages and in those into which other leaf-hoppers were introduced have remained healthy. *Agallia sticticollis* Stål is beyond all doubt the agent of transmission of sugar beet curly top in the Argentine. A complete account of this later work will be published in Revista Industrial y Agricola de Tucumán.

Had *Eutettix tenella* been found here even in small quantities, it would have been tried out in the experiments the same as the other leaf-hoppers. Mr. Chas. F. Henderson, of the U. S. Department of Agriculture, who is studying the parasites of *Eutettix tenella* in South America, made a careful search for the insect in Tucumán this summer but did not find it. He tells me that it is not likely to be found in regions of such heavy rainfall as occurs in this province.

ESTACIÓN ESPERIMENTAL AGRÍCOLA DE TUCUMÁN,
TUCUMÁN, ARGENTINA

ON THE USE OF THE TERMS SAPROPHYTE AND PARASITE

F. L. Stevens and P. A. Young

The terms parasite and saprophyte have long been used in plant pathology. As most authoritatively defined, they signify as follows:

Parasite. Webster's New International Dictionary, 1923: "Biol. A plant or animal living in, on, or with, some other living organism (called its host) at whose expense it obtains its food, shelter, or some other advantage." Webster's Unabridged Dictionary, 1894: "(a) (Bot.) A plant obtaining nourishment immediately from other plants to which it attaches itself, and whose juices it absorbs; sometimes, but erroneously, called epiphyte. (b) A plant living on or within an animal, and supported at its expense, as many species of fungi of the genus *Torrubia.*" Funk and Wagnalls, 1917: "A living organism, either an animal or a plant, that lives on or in some other living organism, known as its host, from which it derives its nourishment for the whole or a part of its existence, as a louse, tapeworm, mistletoe, or dodder." Century Dictionary: "Those which feed on living organisms are termed parasites."

Saprophyte. Century: "Bot. A plant that grows on decaying vegetable matter." Funk and Wagnalls, 1917: "An organism that lives upon dead organic matter, as certain fungous or other plants without chlorophyll, various bacteria, etc." Webster, 1923: "Biol. Any organism living on dead or decaying organic matter. . . . "

These current, popular definitions of the dictionaries do not coincide with technical usage. Thus, the entomologists do not regard insects feeding on or in plants as parasites, nor are mosquitoes or horse flies parasites, in their usage, because they are not constantly attached to their hosts.

The qualifying terms obligate and facultative were introduced with obvious significance: obligate parasites deriving food only from living organisms; facultative, sometimes from living organisms.

There is daily need in plant pathology of terms to designate several categories of parasitism which do not coincide precisely with any of those mentioned above, and for which there are no terms in existence or at least in general use. A few examples representative of large classes of parasites are as follows:

A. An organism like *Venturia inaequalis* is strictly saprophytic during its entire ascigerous period, which is about half of the year. During the other half of the year it is naturally parasitic, yet it germinates *in vitro* and grows well in non-living culture media. Is *Venturia inaequalis* therefore a saprophyte? Is *Fusicladium dendriticum* an obligate parasite?

B. The converse of group "A" (hypothetical): the ascigerous stage is parasitic and the conidial stage is saprophytic.

C. *Ustilago zeae* and *U. avenae* are usually called obligate parasites; yet their sporidia sometimes grow indefinitely as saprophytes and therefore cannot be ignored. Are they then obligate parasites, facultative saprophytes, or just saprophytes?

D. A vast number of pathogenic fungi do not at all, or for only a brief period in their tenancy of the host plant, live in the living tissues of the hosts; on the contrary, they send ahead a sapper van of toxins or enzymes that kills the host cells before the arrival of the fungus. Examples are *Phytophthora infestans, Botrytis,* and numerous other fungi. Are these to be classed as saprophytes?

E. Many fungi possess a mycelium that wanders over the surfaces of the leaves or in the middle lamellae, and sends haustoria into the living cells. Even the haustoria do not attack living protoplasm in many cases, although they may penetrate it to reach the non-living cell contents. Are the powdery and downy mildews really parasites?

F. *Fusarium* gains access to plant tracheae, for example, through a hydathode or a root wound. It multiplies in the non-living contents of the tracheae and causes the death of the host, although it may spend no part of its life in the living tissues of the host. *Pseudomonas campestris* also exemplifies this phenomenon. Are these organisms parasites at all? Consideration of the preceding cases leads to the following conclusions:

Organisms whose presence causes diseases in plants, even though they do not directly attack living plant parts, may properly be called pathogens. (The use of the final "e" in pathogene is no more necessary here than it is in the correlative terms oxygen, hydrogen, etc. Murray's Dictionary gives the spelling as "pathogen," although the Standard Dictionary gives it only as "pathogene.")

But can all these organisms under current, standard, accepted definitions be termed parasites? Indeed, it would appear that few organisms other than the Chytridiales are strict parasites. Shall the definition of parasite be modified or new terms be introduced? We favor the former solution and suggest the following definitions: A parasite is an organism which lives in or is attached to some other species of living organism from the living matter of which it secures part or all of its food materials. A saprophyte is an organism which secures its food from dead organic matter.

Tentatively accepting these definitions, we may denominate as natural parasites such organisms as are parasites in nature, but which can be grown artificially on agar, in culture media in tubes, etc.; or these organisms may be directly designated as artificial saprophytes, or, indeed, as natural parasites (artificial saprophytes). Obligate parasite should mean, as it does now

in definition though not in usage, that an organism is a natural obligate parasite with no artificial saprophytism. A correlative group is artificial parasites (natural saprophytes).

Organisms that are parasitic in both perfect and imperfect stages may be called *totoparasites*. The name *tropoparasite* may be applied to those organisms in which the conidial stage is parasitic and the ascigerous stage is saprophytic. If desirable for clearness, the term *teleutoparasitic* might be used to designate organisms that are parasitic in the perfect stage, and *deuteroparasitic* might be applied to those that are parasitic in the conidial stage.

One other group must be considered: Organisms sometimes parasitic that may in a given stage live as saprophytes, in the soil, for example, or within living host plants. Examples of such are *Fusarium* and various bacteria. Obviously these organisms are readily classified under the old term facultative parasite.

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PHYTOPATHOLOGICAL NOTES

Beef Infusion Versus Beef Extract Media. The conviction has recently been forced upon the writer that not all plant pathologists realize the differences between beef extract media and beef infusion media. Beef extract is now standard in many laboratories, but much early work was done with beef infusion and some pathologists still prefer it for routine work.

Since until recent years Fuller's scale was used to record acidity, this must be used in comparing present work with that of earlier workers. Quirk and Fawcett[1] have shown very clearly in their paper—which, because of its place of publication, probably has not reached the attention of all pathologists—that Fuller's scale values in the two media represent vastly different pH values, and that pH values determine growth. While in the highly buffered beef infusion media + 32 Fuller's scale with a pH value of 5.2 represents the limit of acid toleration for many pathogenes, the same pH in the unbuffered beef extract media is only + 13 Fuller's scale. A worker comparing the toleration of acid by *Bact. pruni* in beef extract media did not understand why it would not grow at + 28 as recorded by an earlier worker, who doubtless used infusion media. The present writer grew the organism in question in extract and infusion media for comparison; the limit of growth was found to be + 29 (pH 5.3) in infusion media but + 13 (pH 5.3) in extract media.

Another difference between the two media is their influence on the formation of green fluorescence. Here again a pathologist finding no green coloring in his beef extract cultures of *Bact. citriputeale* explained the fact that an earlier worker always found green fluorescence with the same organism by supposing that it was a difference in strains of the organism. With this organism the writer tested the two media and found that the greening occurred on beef infusion, not on beef extract.

Other organisms recorded as green fluorescent were then tested on agar slants of the two media with various pH values. The organisms used were *Bact. marginatum, Bact. tabacum, Bact. syringae, Bact. vignae* and *Bact. delphinii.* The following results were obtained.

On beef extract peptone agar a slight greening which ordinarily would go unnoticed was observed with all the organisms in pH 8.0 to pH 8.5 which is beyond the range usually used in routine work, but no greening either above or below this. On the other hand, on beef infusion peptone agar with all the organisms used, green fluorescence was very evident promptly and continuously in pH 6.5 to pH 9.0, strongest in pH 7.0 to pH 8.0.

[1] Quirk, Agnes J., and Edna H. Fawcett. Hydrogen-ion concentration vs. titratable acidity in culture mediums. Jour. Infectious Diseases 33: 1–59. 1923.

With one organism, a strain of *Bact. syringae*, a decided surface wrinkling occurred constantly on beef infusion agar, while on beef extract agar the surface of colonies was always smooth.

It is important, therefore, for all workers to state which medium they have used. Moreover, even though beef extract is used as the standard medium, beef infusion agar is a better differential medium for green fluorescence and for some other cultural characters.—MARY K. BRYAN, Laboratory of Plant Pathology, Bureau of Plant Industry, U. S. Dept. of Agriculture.

An Emendation of the Description of Ophiobolus heterostrophus. In my account[1] of *Ophiobolus heterostrophus*, a fungus causing a leafspot of maize in many of the warmer regions of the world, the filamentous ascospores were described in part as being typically four in number and disposed in multiple heterostrophic helicoid arrangement. In connection with experimental work subsequently undertaken to determine the conditions involved in the development of the ascigerous stage, some lots of material were obtained in which each ascus regularly contained eight spores, the larger number of spores entailing a corresponding increase in the volume of the containing structures. In somewhat exceptional instances, also, a reversal in direction of rotation of the spores was observed in the basal portion of the ascus, although such cases were hardly frequent enough to render the specific name of the parasite generally inappropriate. While reversal in direction of rotation may probably best be interpreted as a casual irregularity, the inconstancy in number of ascospores would seem more likely to be related to variation in basic developmental processes. Whatever explanation may be attached to these features as a result of further study, they are pointed out in the present note mainly for the purpose of emending the morphological definition of the species. The emendation is of interest especially because the ascigerous stage under consideration is the only one hitherto recorded as associated with any species of that type of *Helminthosporium* which is distinguished by the bipolar germination of ellipsoidal conidia.—CHARLES DRECHSLER, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Powdery mildew of muskmelon.—Dr. I. C. Jagger, in Phytopath. **16**: 1009–1010. 1926, reports on "Powdery mildew of muskmelon in the Imperial Valley of California in 1925" and states that perithecia of the fungus have not been found. The writer of the present note just happened to read S. U. Shambel's note on "*Sphaerotheca fuliginea* (Schecht.) Pall. on Muskmelon" in the Russian journal "Diseases of Plants," 51–52, 1926. Shambel

[1] Drechsler, C. Leafspot of maize caused by *Ophiobolus heterostrophus*, n. sp., the ascigerous stage of a *Helminthosporium* exhibiting bipolar germination. Jour. Agr. Res. **31**: 701–726. 1925.

reports that some immature perithecia of muskmelon powdery mildew were found in 1923 in southeast Russia and numerous mature perithecia in 1925, and that the fungus was determined to be *Sphaerotheca fuliginea*.—C. D. SHERBAKOFF, University of Tennessee Experiment Station, Knoxville, Tennessee.

The summer meeting of the American Phytopathological Society.—The 1927 annual summer field meeting and tour will be held in northern Ohio, August 16–19. It is planned to assemble at the Ohio Agricultural Experiment Station and spend Tuesday forenoon, August 16, visiting the experimental disease plots on vegetables and fruits. The afternoon of the first day will be spent in the extensive muck regions around Lodi and Willard. These mucks have been under continuous cultivation for over 25 years, and diseases of all the crops grown there are abundant and severe.

The second day of the tour will be spent in the vegetable and small fruit sections around Clyde, and Catawba Island. Experimental disease plots of tomatoes, potatoes, pickles, sweet corn, peas, sugar beets, raspberries, grapes, and peaches will be visited. In the evening of the second day a boat trip has been arranged for a visit to (a) Kelly's Island, where the experimental plots for the control of mildew and black rot will be studied, and to (b) South Bass Island, where the party will have dinner and lodging.

The third day the tour will continue along the lake to Cleveland. The numerous stops will include a study of raspberry, cherry, and vegetable diseases.

The fourth day the group will be split up according to interests. One party will visit the extensive raspberry propagating region southeast of Cleveland. This part of the tour will be under the supervision of Mr. Wilcox, who will show the disease-free raspberry plantings. The second group will visit the Painesville nurseries and Dr. Bingham's orchard, the largest apple orchard east of the Rockies.

The third group will accompany Mr. Tilford to the large potato section southeast of Cleveland.

The tour has been arranged to show as many of the experimental disease plots on fruits and vegetables as possible. It will include a great variety of diseases on these crops. Many points of natural and historical interest will be included, such as Green Springs, the Blue Hole, the islands of Lake Erie, and Perry's monument.

Automobile space will be arranged at Wooster for all not driving. An attempt will be made to send all members of the Society a letter explaining the tour. Should you fail to receive this, however, please feel that you are invited and that the Ohio pathologists will take care of you.—H. V. YOUNG, Chairman of Committee on Local Arrangements, Wooster, Ohio.